Европейски съюз

ЕВРОПЕЙСКИ СОЦИАЛЕН ФОНД 2007 – 2013
МИНИСТЕРСТВО НА ОБРАЗОВАНИЕТО И НАУКАТА
ОПЕРАТИВНА ПРОГРАМА „РАЗВИТИЕ НА ЧОВЕШКИТЕ РЕСУРСИ"

BG051PO001-3.3.06 -0059

Европейски социален фонд

ФУНДАМЕНТАЛНО И ПРИЛОЖНО ОБУЧЕНИЕ
НА ДОКТОРАНТИ, ПОСТДОКТОРАНТИ,
СПЕЦИАЛИЗАНТИ И МЛАДИ УЧЕНИ
В ИНТЕРДИСЦИПЛИНАРНИ БИОЛОГИЧНИ НАПРАВЛЕНИЯ
И ИНОВАЦИОННИ БИОТЕХНОЛОГИИ.

Бенефициент:
Институт по биология и имунология на размножаването "Акад. Кирил Братанов"

Партньори:

Софийски Университет „Св. Климент Охридски", Биологически Факултет
Химикотехнологичен и металургичен университет, катедра „Биотехнология"
Проген ООД

7

Образец № 3

ДО ИБИР – БАН
бул. „Цариградско шосе" № 73
гр. София

ТЕХНИЧЕСКА ОФЕРТА

За участие в открита процедура за възлагане на обществена поръчка с предмет:

«Доставка на материали /реактиви/ и консумативи по обособени позиции»

ЗА ОБОСОБЕНА ПОЗИЦИЯ № **7** и име – **Първични антитела за имунохистохимия**

Настоящата оферта е подадена от: **БИОПЛАСТ ЕООД** */наименование на участника/,* *ЕИК/БУЛСТАТ* **200797338**;

УВАЖАЕМА ГОСПОЖО ДИРЕКТОР,

Заличени подписи - чл.2, ал.1 от ЗЗЛД

Проектът се осъществява с финансовата подкрепа на Оперативна програма „Развитие на човешките ресурси" 2007-2013, съфинансирана от Европейския съюз чрез "Европейския социален фонд"

Заличен печат - чл.37, ал. 1 от ЗЗК - търговска тайна;
Заличен подпис - чл.2, ал.1 от ЗЗЛД

1. С настоящето представяме нашата техническа оферта за обособена позиция **№ 7 и име Първични антитела за имунохистохимия** *(офертата се изготвя за всяка обособена позиция по отделно)* от поръчката.

Предлагаме следните артикули за обособената позиция, по която участвуваме, като прилагаме попълнена таблица, доказваща съответствието на предлаганите от нас артикули с изискванията на Възложителя:

Заличени подписи - чл.2, ал.1 от ЗЗЛД

Заличен печат - чл.37, ал. 1 от ЗЗК - търговска тайна;
Заличен подпис - чл.2, ал.1 от ЗЗЛД

Проектът се осъществява с финансовата подкрепа на Оперативна програма „Развитие на човешките ресурси" 2007-2013, съфинансирана от Европейския съюз чрез "Европейския социален фонд"

(1)	(2)	(3)	(4)	(5)	(6)	(7)
№ по ред	Номер от ОП	Наименование	Единица мярка	Коли-чество	Предложение на участника, включващо технически характеристики на артикула, съобразно изискванията на техническата спецификация на Възложителя	Предложение на участника, включващо каталожен или партиден номер на артикула, даден от производителя на артикула и име на производителя на артикула (тази колона се попълва задължително само за обособени позиции № 1 – 38)
	ОП Р-7	Обособена позиция № 7 – Име – Първични антитела за имунохистохимия				
70	ОП Р-7-1	Мише моноклонално антитяло срещу Тирозин хидроксилаза (клас IgM); подходящо за имуноблот; да реагира с мишка, плъх и човек; разфасовка – не по-малко от 200 µg в 1 ml	опаковка	1	Мише моноклонално антитяло срещу Тирозин хидроксилаза (клас IgM); подходящо за имуноблот; реагира с мишка, плъх и човек; разфасовка – 200 µg в 1 ml	sc-374048 Santa Cruz Biotechnology, Inc.
71	ОП Р-7-2	Заешко анти-мише IgM - FITC; разфасовка - не по-малко от 200 µg в 0.5 ml	опаковка	1	Заешко анти-мише IgM - FITC; разфасовка - 200 µg в 0.5 ml	sc-358950 Santa Cruz Biotechnology, Inc.
72	ОП Р-7-3	Мише моноклонално антитяло срещу Synaptotagmin I (клас IgG); подходящо за имуноблот; да реагира с мишка, плъх и човек; разфасовка - не по-малко от 200 µg в 1 ml	опаковка	1	Мише моноклонално антитяло срещу Synaptotagmin I (клас IgG); подходящо за имуноблот; реагира с мишка, плъх и човек; разфасовка - 200 µg в 1 ml	sc-136480 Santa Cruz Biotechnology, Inc.
73	ОП Р-7-4	Заешко поликлонално антитяло срещу AlphaB Crystallin; подходящо за имунохистохимия на парафинови и криостатни срези, имуноблот и имуноцитохимия; да реагира с мишка и човек; разфасовка - 100 µl; концентрация - 1 mg/ml	опаковка	1	Заешко поликлонално антитяло срещу Alpha B Crystallin; подходящо за имунохистохимия на парафинови и криостатни срези, имуноблот и имуноцитохимия; реагира с мишка и човек; разфасовка - 100 µl; концентрация - 1 mg/ml	ab13497 Abcam
74	ОП Р-7-5	Заешко поликлонално антитяло срещу AlphaB Cristallin (phosphor S59); подходящо за имунохистохимия на парафинови и криостатни срези,	опаковка	1	Заешко поликлонално антитяло срещу Alpha B Crystallin (phospho S59); подходящо за имунохистохимия на парафинови и криостатни срези,	ab5577 Abcam

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		имуноблот и имуноцитохимия; да реагира с мишка и човек; разфасовка - 100 µl; концентрация - 1 mg/ml			имуноблот и имуноцитохимия; реагира с мишка и човек; разфасовка - 100 µl; концентрация - 1 mg/ml	
75	ОП Р-7-6	Мише моноклонално антитяло срещу Kisspeptin-1; подходящо за имунохистохимия на парафинови срези; концентрация - 100 µg/ml.	опаковка	1	Мише моноклонално антитяло срещу Kisspeptin-1; подходящо за имунохистохимия на парафинови срези; концентрация - 100 µg/ml.	sc-101246 Santa Cruz Biotechnology, Inc.
76	ОП Р-7-7	Антитяло срещу Мелатонин-1 рецептор, подходящо за имунохистохимия на парафинови срези	опаковка	1	Козе поликлонално антитяло срещу Мелатонин-1В-рецептор; подходящо за имунохистохимия на парафинови срези; разфасовка - 200 µg в 1 ml	sc-13174 Santa Cruz Biotechnology, Inc.
77	ОП Р-7-8	Антитяло и блокиращ реактив за детекция на GDF9 в заешки тъкани, разфасовка от 100 мкл	опаковка	1	Набор за имунохистохимия, съдържащ: 1. Заешко поликлонално антитяло срещу GDF-9 2. Блокиращ реактив при използването на заешки антитела върху заешка тъкан – 15 ml	ABIN734543 Antibodies-Online RTR015 ScyTek, Inc.
78	ОП Р-7-9	Антитяло и блокиращ реактив за детекция на BMP15 в заешки тъкани, разфасовка от 100 мкл	опаковка	1	Набор за имунохистохимия, съдържащ: 1. Заешко поликлонално антитяло срещу BMP-15 2. Блокиращ реактив при използването на заешки антитела върху заешка тъкан – 15 ml	ABIN718841 Antibodies-Online RTR015 ScyTek, Inc.
79	ОП Р-7-10	Антитяло срещу Ki-67; подходящо за флоуцитометрия, имунохистохимия, имунофлуоресценция и имуноблот;	опаковка	1	Козе поликлонално антитяло срещу Ki-67; подходящо за флоуцитометрия, имунохистохимия, имунофлуоресценция и имуноблот;	sc-7846 Santa Cruz Biotechnology, Inc.
80	ОП Р-7-11	Моноклонално антитяло срещу афлатоксин	опаковка	1	Моноклонално антитяло срещу Aflatoxin antibody [ATB]	ab1985 Abcam
81	ОП Р-7-12	Антитяло срещу каспаза 3, апоптоза-свързана цистеинова пептидаза	опаковка	1	Заешко моноклонално антитяло срещу Caspase 3, Apoptosis-Related Cysteine Peptidase (CASP3)	ABIN189202 Antibodies-Online
82	ОП Р-7-13	Антитяло срещу тубулин	опаковка	1	Tubulin (TUB) antibody	ABIN934110 Antibodies-Online
83	ОП Р-7-14	Поликлонално антитяло срещу MUC1; подходящо за имунохистохимия, имунофлуоресценция и имуноблот; разфасовка от 0.05 гр.	опаковка	1	Заешко поликлонално антитяло срещу Mucin 1, Cell Surface Associated (MUC1) (C-Term); подходящо за имунохистохимия, имунофлуоресценция и имуноблот; разфасовка от 0.05 гр.	ABIN310153 Antibodies-Online
84	ОП Р-7-15	Моноклонално антитяло срещу Bestrophin	опаковка	2	Мише моноклонално антитяло срещу	ABIN152509

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		1 (клон E6-6); подходящо за имунохистохимия, имуноблот, имунопреципитация и имунофлуоресценция; разфасовка от 0.1 мл.			Bestrophin 1 (BEST1) (клон E6-6); подходящо за имунохистохимия, имуноблот, имунопреципитация и имунофлуоресценция; разфасовка от 0.1 мл.	Antibodies-Online
85	ОП Р-7-16	Антитяло срещу beta-catenin	опаковка	1	Заешко поликлонално антитяло срещу beta-catenin	sc-7199 Santa Cruz Biotechnology, Inc.
86	ОП Р-7-17	Поликлонално антитяло срещу p-ERK 1/2 (Thr202-Tyr204); подходящо за имунохистохимия, имунофлуоресценция, имунопреципитация и имуноблот; разфасовка - не по-малко от 200 µg в 1 ml	опаковка	1	Козе поликлонално антитяло срещу p-ERK 1/2 Antibody (Thr 202/Tyr 204); подходящо за имунохистохимия, имунофлуоресценция, мунопреципитация и имуноблот; разфасовка - 200 µg в 1 ml	sc-16982 Santa Cruz Biotechnology, Inc.
87	ОП Р-7-18	Блокиращ пептид за p-ERK 1/2 (Thr202-Tyr204) антитяло; разфасовка - не по-малко от 100 µg/0.5 ml	опаковка	1	Блокиращ пептид за поликлонално антитяло срещу p-ERK 1/2 (Thr 202/ Tyr 204); разфасовка - 100 µg/0.5 ml	sc-16982 P Santa Cruz Biotechnology, Inc.
88	ОП Р-7-19	Козе поликлонално антитяло срещу Мелатонин - 1В рецептор; подходящо за имунофлуоресценция, имунопреципитация и имуноблот; разфасовка - не по-малко от 200 µg в 1 ml	опаковка	1	Козе поликлонално антитяло срещу MEL-1B-R; подходящо за имунофлуоресценция, имунопреципитация и имуноблот; разфасовка - 200 µg в 1 ml	sc-13174 Santa Cruz Biotechnology, Inc.
89	ОП Р-7-20	Блокиращ пептид за Мелатонин - 1В рецептор антитяло; разфасовка - не по-малко от 100 µg/0.5 ml	опаковка	1	Блокиращ пептид за поликлонално антитяло срещу MEL-1B-R; разфасовка - 100 µg/0.5 ml	sc-13174 P Santa Cruz Biotechnology, Inc.
90	ОП Р-7-21	Поликлонално антитяло срещу CASPASE 3 (CPP32); разфасовка - 0.2 мл.	опаковка	1	Заешко поликлонално антитялос срещу Caspase 3, Apoptosis-Related Cysteine Peptidase (CASP3) - CPP32	ABIN346985 Antibodies-Online
91	ОП Р-7-22	Козе поликлонално антитяло срещу LOX-1; подходящо за имуноблот; разфасовка от 100 µl	опаковка	1	Козе поликлонално антитяло срещу LOX-1; подходящо за имуноблот; разфасовка от 100 µl	3659-100 BioVision
92	ОП Р-7-23	Заешко поликлонално антитяло срещу TLR4; да разпознава епитоп в 242-321 аминокиселинни последователности; подходящо за имунофлуоресценция, имунопреципитация и имуноблот; разфасовка - не по-малко от 200 µg в 1 ml	опаковка	1	Заешко поликлонално антитяло срещу TLR4; да разпознава епитоп в 242-321 аминокиселинни последователности; подходящо за имунофлуоресценция, имунопреципитация и имуноблот; разфасовка - 200 µg в 1 ml	sc-10741 Santa Cruz Biotechnology, Inc.
93	ОП Р-7-24	Антитяло срещу Vinculin; да реагира с мишка; подходящо за имунофлуоресценция	опаковка	1	Козе поликлонално антитяло срещу vinculin; реагира с мишка; подходящо за имунофлуоресценция	sc-7649 Santa Cruz Biotechnology, Inc.
94	ОП Р-7-25	Моноклонално антитяло срещу Integrin beta 1 (активирана форма) (клон 9EG7);	милилитър	1	Плъше моноклонално антитяло срещу Integrin beta 1 (ITGB1) (активирана форма)	ABIN967378 Antibodies-Online

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		подходящо за имунохимия; да реагира с човек и мишка			(клон 9EG7); подходящо за имунохимия; реагира с човек и мишка	
95	ОП Р-7-26	Антитяло срещу LOX-1; разфасовка от 100 µg	опаковка	1	Заешко поликлонално антитяло срещу Oxidized Low Density Lipoprotein (Lectin-Like) Receptor 1 (OLR1) (N-Term); разфасовка от 100 µg	ABIN223339 Antibodies-Online
96	ОП Р-7-27	Антитяло срещу TLR4; разфасовка - не по-малко от 200 µg в 1 ml	опаковка	1	Заешко поликлонално антитяло срещу TLR4; разфасовка - 200 µg в 1 ml	sc-10741 Santa Cruz Biotechnology, Inc.

Забележка: Колони (1) – (5) от таблицата се попълват съгласно Техническата Спецификация на Възложителя - Приложение № 1 от документацията за участие – без да се променят !!.

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2. Срокът за изпълнение на доставки по обособената позиция, след получаване на възлагателно писмо от Възложителя е до 15 календарни дни (*минимум 1 ден и не повече от 45 календарни дни. При непопълване от участника се приема 45 кал. дни).*

3. За обособени позиции № 1 – 38: Остатъчният срок на годност на предложените с офертата стоки към датата на доставка ще бъде 10 месеца (*не по-малко от 9 месеца, където е приложимо*). Под остатъчен срок на годност се има предвид времето за което реактивът е годен за употреба след доставка в сградата на Възложителя.

4. Ако бъдем избрани за изпълнител се ангажираме да осигурим предложените с офертата стоки за целия срок на договора. В случай, че поради непредвидени обстоятелства същите бъдат спрени от производство се ангажираме да предложим продукт с еквивалентни или сходни характеристики.

5. Осигуряваме следното време за реакция, при подадена рекламация на артикул от страна на Възложителя, съгласно условията на договора: 1 дни *(минимум 1 и максимум 14 календарни дни. При непопълване от участника се приема 14 кал. дни).* В посочения срок ще отговорим на Възложителя обосновано в писмен вид дали приемаме или отхвърляме рекламацията.

6. Стоките (с изключение на рециклирани тонери) ще се доставят в оригинална, ненарушена опаковка на производителя, при спазване на условията на производителя за транспорт и съхранение на артикула.

7. При доставка, стоките ще бъдат придружени от приложимите за случая сертификати и документи, издадени от производителя и/или контролиращи организации.

8. Подаването на настоящата техническа оферта удостоверява безусловното приемане от наша страна на всички изисквания и задължения, поставени от Възложителя в провежданата процедура за съответната обособена позиция.

! ВАЖНО: Ако участникът подава оферта за повече от една обособена позиция, плик № 2, т.е. Техническа оферта се представя за всяка обособена позиция по отделно в отделен плик № 2 и се надписва по следния начин:

„Име на участника: .....
Предложение за изпълнение на поръчката по обособена позиция № ....”

Дата 8.08.2014 год.
гр. София

..... (
/ подпис, печат

Заличен печат - чл.37, ал. 1 от ЗЗК - търговска тайна;
Заличен подпис - чл.2, ал.1 от ЗЗЛД

Упълномощен да подпише предложението за и от името на ..... *(изписва се името на участника)*
.....
(изписва се името на упълномощеното лице и длъжността, като в случай, че това не е законния представител на участника се прилага нотариално заверено пълномощно).

Заличени подписи - чл.2, ал.1 от ЗЗЛД

Проектът се осъществява с финансовата подкрепа на Оперативна програма „Развитие на човешките ресурси” 2007-2013, съфинансирана от Европейския съюз чрез “Европейския социален фонд“

БИОПЛАСТ ЕООД

1164 София , кв. Лозенец , ул. „ Златовръх „ № 63
Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна
Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна

Отговор

7

По отношение ваше запитване ,свързано с Липсващи документи и констатирани нередовности по отношение на документите в плик № 1 от офертата за обособени позиции № 7, Заличени текстове, тъй като се отнасят до други обособени

1. Прилагам Списък на документите, съдържащи се в офертата, Административни сведения за участника и попълнения образец на Оферта за участие в процедурата, подписани от управителя Калин Константинов Краев.
2. Прилагам декларация за отсъствие на обстоятелствата по чл. 47, ал. 2 от ЗОП - Образец № 6 от документацията за участие със заявено: „4. Представляваният от мен участник няма задължения по смисъла на чл. 162, ал. 2, т. 1 от Данъчно-осигурителния процесуален кодекс към държавата и към община, установени с влязъл в сила акт на компетентен орган, освен ако е допуснато разсрочване или отсрочване на задълженията, *а в случай че участникът е чуждестранно лице* – няма задължения за данъци или вноски за социалното осигуряване съгласно законодателството на държавата, в която участникът е установен».

По отношение ваше запитване ,свързано с Констатирани Нередовности и Въпроси: По отношение на представените в Плик № 1 брошури, каталози, извадки от брошури, каталози и други документи на производителя, указващи името на производителя на артикула, каталожен или партиден номер на артикула, техническите и функционални характеристики на артикула за обособени позиции 7, Заличени текстове, тъй като се отнасят до други обособени позиции. **прилагам:**

- Преводи на български език на брошурите за артикули от Обособена позиция № 7 – „Първични антитела за имунохистохимия". Позиции 7.18 и 7.20 са пептиди и тяхното описание е в брошурите на антителата 7.17 и 7.19.
- Заличени текстове, тъй като се отнасят до други обособени позиции.
-
-

2. Забележки и Въпроси по брошурите за артикулите от **обособена позиция № 7**:

BIO PLAST БИОПЛАСТ ЕООД

1164 София , кв. Лозенец , ул. „ Златовръх „ № 63
Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна
Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна

2.1. За артикул 7.7 –потвърждаваме ,че антитялото е приложимо за имунохистохимия на парафинови срези.Като доказателство прилагаме две резюмета от статии.

2.2. За артикул 7.10 – в брошурата на английски език не е отбелязано дали антитялото срещу Ki-67 се използва за имунохистохимия, както изисква заданието. Отговор:Приложена е публикация ,от която е видно ,че антиялото работи на имунохистохимия.

2.3. За артикул 7.15 – в брошурата на английски език не е отбелязано дали моноклоналното антитяло срещу Bestrophin 1 клон E6-6 е подходящо за имунохистохимия и имунопреципитация, което се изисква от заданието; В брошурата не е отбелязано, че предлаганото антитяло е приложимо за имунофлуоресценция.Отговор :Приложени са пълната брошура на съответното антияло и нейният превод.

2.4. За артикул 7.18 - няма приложена брошура с превод на български език за блокиращ пептид за p-ERK ½, Thr202-Tyr2014, антитяло, концентрация не по-малка от 100µg в 0.5 ml. Участникът да представи брошура или извадка от каталог на производителя за каталожен номер sc16982-P с превод на български език, съгласно изискванията на т. 7.3.2.3 от документацията за участие – с технически и функционални характеристики на артикула.Отговор:Тъй като това са пептиди ,срещу които са получени съответните антитела,тяхната характеристика е дадена в съответната брошура на антителата със съответните количества и концентрация.Производителят не предоставя брошура за пептидите,срещу които са произведени антителата.

2.5. За артикул 7.20 – няма приложена брошура с превод на български език за блокиращ пептид за Мелатонин - 1B рецептор антитяло; разфасовка - не по-малко от 100 µg/0.5 ml. Участникът да представи брошура или извадка от каталог на производителя за каталожен номер sc13174-P с превод на български език, съгласно изискванията на т. 7.3.2.3 от документацията за участие - с технически и функционални характеристики на артикула. Отговор:Тъй като това са пептиди ,срещу които са получени съответните антитела,тяхната характеристика е дадена в съответната брошура на антителата със съответните количества и концентрация.Производителят не предоставя брошура за пептидите,срещу които са произведени антителата.

2.6. За артикул 7.25 – в брошурата на английски език не е отбелязано дали моноклоналното антитяло срещу Integrin beta 1 може да се използва върху човешки тъкани. Участникът по възможност да представи потвърждение от производителя – с превод на български език, дали предлаганото съгласно брошурата моноклонално антитяло може да се използва върху човешки тъкани. Отговор:Прилагаме публикация ,от която е видно,че антитялото показва кръстосана реактивност с човек.

Заличени текстове, тъй като се отнасят до други обособени позиции.

БИОПЛАСТ ЕООД

1164 София , кв. Лозенец , ул. „ Златовръх „ № 63

Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна

Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна

Заличени текстове, тъй като се отнасят до други обособени позиции.

БИОПЛАСТ ЕООД

1164 София , кв. Лозенец , ул. „ Златовръх „ № 63
Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна
Заличени данни - чл.37, ал. 1 от ЗЗК - търговска тайна

Заличени текстове, тъй като се отнасят до други обособени позиции.

Изготвил: Заличени лични данни - чл.2, ал.1 от ЗЗЛД

Управител на БИОПЛАСТ ЕООД

Заличен печат - чл.37, ал. 1 от ЗЗК - търговска тайна;
Заличен подпис - чл.2, ал.1 от ЗЗЛД

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

TH (A-6): sc-374048

Обща информация:	----------
Хромозомна локализация:	Генетичен локус: TH (човек) – 11p15.5; Th (мишка) – 7 F5
Източник:	TH (A-6) е мише моноклонално антитяло, специфично срещу епитоп намиращ се между аминокиселините 500-526 в С-терминалния край на TH от човешки произход.
Продукт:	Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.
Съхранение:	Съхранявайте при 4° С, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.
Приложения:	TH (A-6) се препоръчва за доказване на TH от миши, плъши и човешки произход посредством Имуноблот (начално разреждане 1:100, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000). Подходящо за използване като контролно антитяло за TH siRNA (h): sc-36662, TH siRNA (m): sc-36661, TH shRNA Plasmid (h): sc-36662-SH, TH shRNA Plasmid (m): sc-36661-SH, TH shRNA (h) Lentiviral Particles: sc-36662-V и TH shRNA (m) Lentiviral Particles: sc-36661-V. Молекулна маса на TH: 60 kDa. Положителна контрола: PC-12 клетъчен лизат: sc-2250, PC-12 + NGF клетъчен лизат: sc-3808 или миши мозъчен екстракт: sc-2253.
Препоръчани вторични реактиви:	За постигане на оптимални резултати се препоръчват следните вторични реактиви: 1) Имуноблот: използвайте козе анти-мише IgM-HRP: sc-2064 (диапазон на разреждане: 1:500-1:5,000), TBS Blotto A Блокиращ Реактив: sc-2333 и Имуноблот Луминол Реактив: sc-2048. 2) Имунопреципитация: използвайте Protein L PLUS-Agarose: sc-2336 (0.5 ml agarose/2.0 ml). 3) Имунофлуоресценция: използвайте козе анти-мише IgM-FITC: sc-2082 (диапазон на разреждане: 1:100-1:400) или козе анти-мише IgM-TR: sc-2983 (диапазон на разреждане: 1:100-1:400) с UltraCruz™ Среда за покриване: sc-24941.
Изследователска употреба:	Само за изследователски цели, а не за диагностични процедури

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

Заешки анти-миши IgM-FITC: sc-358950

Обща информация: ----------

Източник: Заешки анти-миши IgM-FITC е преабсорбирано, афинитетно пречистено вторично антитяло получено в заек, срещу миши IgM и конюгирано с FITC (fluorescein isothiocyanate).

Продукт: Всяка виалка съдържа 200 µg заешки IgM (преабсорбирано с миши и човешки IgG) в 0.5 мл. или PBS с 0.2% натриев азид (за IF), или PBS с 0.1% гел и 0.1% натриев азид (за FCM)

Приложения: Заешки анти-миши IgM-FITC се препоръчва за детекция на миши IgM посредством Имунофлуоресценция (начално разреждане 1:100, диапазон на разреждане 1:100-1:400), Имунохистохимия (начално разреждане: 1:100, диапазон на разреждане: 1:100-1:400) и флоуцитометрия (0.5-1 µg на $1x10^6$ клетки).

Препоръчани вторични реактиви:

А. КУЛТИВИРАНИ КЛЕТКИ

- CrystalCruz™ покривни стъкла, 22 x 50 mm, предварително почистени: sc-24975
- CrystalCruz™ Микроскопски стъкла 75 x 25 mm; 72 матирани: sc-24976
- PBS (Фосфатно-буфериран разтвор), прах, 1 пакет: sc-24947
- Формалдехид, 37% формалдехиден разтвор, 25 ml: sc-203049
- Водороден прекис, 30% разтвор, 100 ml: sc-203336

В. ЗАМРАЗЕНИ ТЪКАННИ СРЕЗИ

- Organo/Limonene Mount, нетоксичен, лтернатива на Permount, 100 ml: sc-45087
- UltraCruz™ среда за покриване на срези, на водна основа, 10 ml: sc-24941
- ImmunoHistoMount, среда за покриване на срези на водна основа, 30 ml: sc-45086
- Immuno In Situ Mount, за употреба с ин ситу хибридизация, 30 ml: sc-45088

С. ФИКСИРАНИ ВЪВ ФОРМАЛИН И ВКЛЮЧЕНИ В ПАРАФИН ТЪКАННИ СРЕЗИ

- Парафин, за подготовка на тъканни проби за оцветяване, 500 g: sc-286633
- Ксилол, смесени изомери с етилбензен, 500 ml: sc-237422
- Хематоксилин по Gill's #2; ядрено контраоцветяване, 100 ml: sc-24973

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

Synaptotagmin I (15): sc-136480

Обща информация:	---
Хромозомна локализация:	Генетичен локус: SYT1 (човек) - 12q21.2; Syt1 (мишка) - 10 D1.
Източник:	Synaptotagmin I (15) е мише моноклонално антитяло, получено срещу аминокиселините 250-259 на Synaptotagmin I от човешки произход.
Продукт:	Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.
Изследователска употреба:	Само за изследователски цели, а не за диагностични процедури
Приложения:	Synaptotagmin I (15) се препоръчва за доказване на Synaptotagmin I (15) от миши, плъши и човешки произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000). Подходящо за използване като контролно антитяло за Synaptotagmin I (15) siRNA (h): sc-41310, Synaptotagmin I (15) siRNA (m): sc-41311, Synaptotagmin I (15) shRNA Plasmid (h): sc-41310-SH, Synaptotagmin I (15) shRNA Plasmid (m): sc-41311-SH, Synaptotagmin I (15) shRNA (h) Lentiviral Particles: sc-41310-V и Synaptotagmin I (15) shRNA (m) Lentiviral Particles: sc-41311-V. Молекулна маса на Synaptotagmin I (15): 40/65 kDa. Положителна контрола: екстракт от мозък на плъх: sc-2392
Препоръчани вторични реактиви:	За постигане на оптимални резултати се препоръчват следните вторични реактиви: 1) Имуноблот: използвайте козе анти-мише IgG-HRP: sc-2005 (диапазон на разреждане: 1:2000-1:32,000) или Cruz Marker™ съвместим кози анти-миши IgG-HRP: sc-2031 (диапазон на разреждане: 1:2000-1:5000), Cruz Marker™ Стандарт за молекулна маса : sc-2035, TBS Blotto A Блокиращ Реактив: sc-2333 и Имуноблот Луминол Реактив: sc-2048.
Съхранение:	Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Поз.7.4.

Превод от английски език

Лого на Abcam Информационен лист

Анти-Alpha B Crystallin антитяло

Наименование на продукта	Анти-Alpha B Crystallin антитяло
Код на продукта	ab13497
Обем	100 µl
Описание	Заешко поликлонално срещу Alpha B Crystallin
Специфичност	Това антитяло не показва кръстосана реактивност нито с beta Crystallin нито с alpha A Crystallin.
Тествани приложения	IHC-Fr, WB, ICC, IP, IHC-P
Видова реактивност	**Реагира с:** Мишка, Плъх, Пиле, Крава, Човек **Предполага се, че работи с:** Заек, Хамстер
Имуноген	Синтетичен пептид: REEKPAVTAAPKK конюгиран към KLH, кореспондиращ с АК 163-175 на човешки alpha B Crystallin.

Свойства

Форма	Течна
Инструкции за съхранение	Съхранявайте при +4°C за кратко (1-2 седмици). Разфасовайте и съхранявайте при -20°C или -80°C. Избягвайте повторно замразяване/размразяване.
Буфер за съхранение	Консерванг: няма Съставки: цял серум
Чистота	Цял антисерум
Клоналност	Поликлонално
Подтип	IgG

Приложения

IHC-Fr	Използвайте в концентрация в зависимост от метода
WB	1/1000 - 1/2000. Доказва ивица с приблизителна молекулна маса 22 kDa (предполагаема мол.маса : 20 kDa).
ICC	1/200
IP	Използвайте в концентрация 5 µg/ml.
IHC-P	Използвайте в концентрация в зависимост от метода

Поз.7.5.

Превод от английски език

Лого на Abcam

Информационен лист

Анти- Alpha B Crystallin (phospho S59) антитяло

Наименование на продукта	Анти- Alpha B Crystallin (phospho S59) антитяло
Код на продукта	ab5577
Обем	100 µg
Описание	Заешко поликлонално срещу Alpha B Crystallin (phospho S59)
Тествани приложения	IHC-Fr, WB, ICC/IF, IHC-P
Видова реактивност	**Реагира с:** Мишка, Плъх, Крава, Човек **Предполага се, че работи с:** Овца, Заек, Хамстер, Свиня, Маймуна
Имуноген	Синтетичен пептид, кореспондиращ с човешки Alpha B Crystallin АК 54-64 (phospho S59) Секвенция: FLRAPSWIDTG

Свойства

Форма	Течна
Инструкции за съхранение	Съхранявайте при +4°C за кратко (1-2 седмици). Разфасовайте и съхранявайте при -20°C или -80°C. Избягвайте повторно замразяване/размразяване.
Буфер за съхранение	Консервант: 0.05% натриев азид Съставки: 99% PBS, 3% BSA
Концентрация	100 µg при 1 mg/ml
Чистота	Афинитетно пречистено
Клоналност	Поликлонално
Подтип	IgG

Приложения

IHC-Fr	Използвайте в концентрация в зависимост от метода. PubMed: 21126175
WB	Използвайте в концентрация 5 µg/ml.Доказва ивица с приблизителна молекулна маса 20 kDa.
ICC/IF	Използвайте в концентрация 8 µg/ml.
IHC-P	Използвайте в концентрация 8 µg/ml.

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

KiSS-1 (24-Q): sc-101246

Обща информация: ----------

Хромозомна локализация: Генетичен локус: KISS1 (човек) - 1q32.1.

Източник: KiSS-1 (24-Q) е мише моноклонално антитяло, специфично срещу рекомбинантен KiSS-1 от човешки произход.

Продукт: Всяка виалка съдържа 100 µg IgG_{2a} в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Приложения: KiSS-1 (24-Q) се препоръчва за доказване на KiSS-1 от човешки произход посредством имунофлуоресценция, имунохистохимия (включително парафинови срези) (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Подходящо за използване като контролно антитяло за KiSS-1 siRNA (h): sc-37443.

Молекулна маса на KiSS-1: 15 kDa.

Положителна контрола: PC-12 клетъчен лизат: sc-2250, PC-12 + NGF клетъчен лизат: sc-3808 или миши мозъчен екстракт: sc-2253.

Препоръчани вторични реактиви: За постигане на оптимални резултати се препоръчват следните вторични реактиви: 1) Имунофлуоресценция: използвайте козе анти-мише IgG-FITC: sc-2010 (диапазон на разреждане: 1:100-1:400) или козе анти-мише IgG-TR: sc-2781 (диапазон на разреждане: 1:100-1:400) с with UltraCruz™ среда за покриване: sc-24941.
2) Имунохистохимия: използвайте ImmunoCruz™: sc-2050 или ABC: sc-2017 мише IgG оцветяваща система

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

MEL-1B-R (H-18): sc-13174

Обща информация: ----------

Хромозомна локализация: Генетичен локус: MTNR1B (човек) - 11q14.3.

Източник: MEL-1B-R (H-18) е афинитетно пречистено козе поликлонално антитяло получено срещу пептид във вътрешния регион на MEL-1B-R от човешки произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

За конкурентни изследвания е наличен блокиращ пептид - sc-13174 P, (100 µg пептид в 0.5 ml PBS съдържащ < 0.1% натриев азид и 0.2% BSA).

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Приложения: MEL-1B-R (H-18) се препоръчва за доказване на MEL-1B-R от човешки произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Подходящо за използване като контролно антитяло за MEL-1B-R siRNA (h): sc-40114, MEL-1B-R shRNA Plasmid (h): sc-40114-SH и MEL-1B-R shRNA (h) Lentiviral Particles: sc-40114-V..

Молекулна маса на MEL-1B-R: 36 kDa.

Положителна контрола: COLO 205 тотален клетъчен лизат: sc-364177.

Препоръчани вторични реактиви: За постигане на оптимални резултати се препоръчват следните вторични реактиви:
1) Имуноблот: използвайте матарешко анти-козе IgG-HRP: sc-2020 (диапазон на разреждане: 1:2000-1:100,000) или Cruz Marker™ съвместимо магарешко анти-козе IgG-HRP: sc-2033 (диапазон на разреждане: 1:2000-1:5000), Cruz Marker™ стандарт за молекулна маса: sc-2035, TBS Blotto A Блокиращ Реактив: sc-2333 и Имуноблот Луминол Реактив: sc-2048.
2) Имунопреципитация: използвайте Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).
3) Имунофлуоресценция: използвайте магарешко анти-козе IgG-FITC: sc-2024 (диапазон на разреждане: 1:100-1:400) или магарешко анти-козе IgG-TR: sc-2783 (диапазон на разреждане: 1:100-1:400) с UltraCruz™ среда за покриване: sc-24941.

7028-7.7

PubMed

Display Settings: Abstract

J Pineal Res. 2008 Aug;45(1):50-60. doi: 10.1111/j.1600-079X.2008.00555.x. Epub 2008 Feb 25.

Human placental trophoblasts synthesize melatonin and express its receptors.

Lanoix D[1], Beghdadi H, Lafond J, Vaillancourt C.

Author information

Abstract

Although the role of melatonin on fetal development has been the subject of a number of studies, little is known about the function of melatonin in the placenta. We previously showed that melatonin receptors are expressed and are functional in JEG-3 and BeWo cell lines, both in vitro models of human trophoblast. Local synthesis of melatonin in placenta has been proposed, but the human placenta's ability to synthesize melatonin de novo has never been studied. The purpose of this study was to investigate the expression [reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis] and activity (radiometric assay) of melatonin synthesizing enzymes, and characterize the expression of the melatoninergic receptors in human term villous trophoblast. The results show that arylalkylamine N-acetyltransferase and hydroxyindole O-methyltransferase melatonin synthesizing enzymes are expressed and active in villous trophoblast as well as in JEG-3 and BeWo placental choriocarcinoma cells. In addition, immunohistochemical analysis demonstrated the presence of MT1, MT2, and retinoid-related orphan nuclear receptor alpha melatonin receptor proteins in both villous cytotrophoblast and syncytiotrophoblast (STB) as well as in endothelial cells surrounding the fetal capillaries and in the villous mesenchymal core. RT-PCR and western blot analysis in primary cultures of human term trophoblast confirmed the expression of all three melatonin receptors in villous cytotrophoblast and STB cells. This study demonstrates for the first time a local synthesis of melatonin and expression of its receptors in human trophoblasts and strongly suggests a paracrine, autocrine, and/or intracrine role for this indolamine in placental function and development as well as in protection from oxidative stress.

PMID:18312298[PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

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PubMed

Display Settings: Abstract

J Pineal Res. 2008 Nov;45(4):403-12. doi: 10.1111/j.1600-079X.2008.00608.x. Epub 2008 Jul 10.

Melatonin as a negative mitogenic hormonal regulator of human prostate epithelial cell growth: potential mechanisms and clinical significance.

Tam CW[1], Chan KW, Liu VW, Pang B, Yao KM, Shiu SY.

Author information

Abstract

Circannual variation in the human serum levels of prostate-specific antigen, a growth marker of the prostate gland, has been reported recently. The present study was conducted to investigate the role of the photoperiodic hormone melatonin (MLT) and its membrane receptors in the modulation of human prostate growth. Expression of MT(1) and MT(2) receptors was detected in benign human prostatic epithelial tissues and RWPE-1 cells. MLT and 2-iodomelatonin inhibited RWPE-1 cell proliferation and up-regulated p27(Kip1) gene and protein expression in the cells. The effects of MLT were blocked by the nonselective MT(1)/MT(2) receptor antagonist luzindole, but were not affected by the selective MT(2) receptor antagonist 4-phenyl-2-propionamidotetraline. Of note, the antiproliferative action of MLT on benign prostate epithelial RWPE-1 cells was effected via increased p27(Kip1) gene transcription through MT(1) receptor-mediated activation of protein kinase A (PKA) and protein kinase C (PKC) in parallel, a signaling process which has previously been demonstrated in 22Rv1 prostate cancer cells. Taken together, the demonstration of the MT(1)/PKA+PKC/p27(Kip1) antiproliferative pathway in benign and malignant prostate epithelial cell lines indicated the potential importance of this MLT receptor-mediated signaling mechanism in growth regulation of the human prostate gland in health and disease. Collectively, our data support the hypothesis that MLT may function as a negative mitogenic hormonal regulator of human prostate epithelial cell growth.

PMID:18637986[PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

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Превод от английски език

Antibodies-Online

Growth Differentiation Factor 9 (GDF9) antibody

Антиген:	Growth Differentiation Factor 9 (GDF9)
Синоними:	Gdf-9, GDF-9, zgc:123030
Реактивност:	Човек, Мишка, Плъх, Пиле, Куче, Свиня, Крава
Гостоприемник:	Заек
Клоналност:	Поликлонално
Конюгат:	Неконюгирано
Приложение:	Имуноблот (WB), ELISA, Имунохистохимия (Парафинови срези) (IHC (p)), Имунофлуоресценция IHC-P (IF (IHC-P))
Каталожен номер:	ABIN734543
Количество:	100 µl
Наличност:	.....

Информация за продукта

Имуноген:	KLH конюгиран синтетичен пептид получен от човешки GDF-9
Изотип:	IgG
Кръстосана реактивност:	Плъх, Пиле, Куче, Свиня, Крава
Пречистване:	пречистен с Protein A колона и пептидна афинитетна хроматография
Общи данни:	----------
Молекулна маса:	50kDa

Детайли на приложение

Бележки за прилагане:	WB (1:100-500), ELISA (1:500-1000), IHC-P (1:100-500), IF (IHCP) (1:100-500)
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Течен
Концентрация:	1 µg/µL
Буфер:	Буфериран разтвор, съдържащ 100 µg/mL BSA, 50 % глицерол и 0.09 % натриев азид
Консерванти:	натриев азид
Съхранение:	-20 °C
Срок на годност:	12 месеца при -20 °C

Поз.7-8-2
Превод от английски език

Лого на ScyTek Laboratories, Inc.

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

Rabbit-To-Rabbit Blocking Reagent

(Блокиращ Реактив за използване на заешки антитела върху заешка тъкан)

Описание на продукта: Rabbit-to-Rabbit реактивът на СкайТек е формулирана да осигури на изследователят система за оцветяване, способна да визуализира заешки антитела върху заешка тъкан. В повечето случаи 30-минутна инкубация с Rabbit-to-Rabbit блока ще елиминира фоновото оцветяване което се причинява от ендогенни имуноглобулини. За оптимални резултати, силно препоръчваме този реактив да се използва в комбинация със УлтраТек Анти-Заешка оцветяваща система на СкайТек

Произход: Коза
Антигенна специфичност: Анти-заешко
Ензимен конюгат: Няма
Хромоген субстрат: Няма

Процедура:

1. Депарафинирайте и рехидратирайте тъканните срези.
2. Ако е необходимо да намалите неспецифичния фон поради наличието на ендогенна пероксидаза,инкубирайте стъклата във водороден прекис за 10-15 мин.
3. Измийте двукратно в буфер.
4. Ако е необходимо, инкубирайте тъканите с ензим.
5. Измийте четирикратно в буфер.
6. Използвайте Супер Блок (СкайТек кат.№ AAA) и инкубирайте за 5 мин на стайна температура за блокиране на неспецифичното фоново оцветяване. **Забележка:** Не превишавайте повече от 10 мин. за да не се намали интензитета на реакцията.
7. Измийте еднократно в буфер.
8. Използвайте Rabbit-to-Rabbit блок и инкубирайте 10-60 мин. Времето за инкубация зависи от количеството на ендогенни имуноглобулини в тъканта.
9. Измийте четирикратно в буфер.
10. Използвайте първично антитяло и инкубирайте съгласно протокола на производителя.
11. Измийте четирикратно в буфер.
12. Използвайте УлтраТек Анти-Поливалент (СкайТек кат.№ ABN) и инкубирайте за 10-20 мин. на стайна температура.
13. Измийте четирикратно в буфер.
14. Използвайте УлтраТек HRP (СкайТек кат.№ ABL) или УлтраТек Алк-Фос (СкайТек кат.№ ABM) и инкубирайте за 10-20 мин. на стайна температура.
15. Измийте четирикратно в буфер.
16. Използвайте подходящ хромоген
17. Контраоцветете и покрийте.

Ръководство за отстраняване на неизправности

Преоцветяване:

1. Концентрацията на първото антитяло е много висока или времето за инкубиране е твърде продължителна.
2. Температурата по време на инкубиране е твърде висока.
3. Времето за инкубиране със свързващото антитяло или стрептавидин-пероксидазата е твърде продължителна.

Неспецифично фоново оцветяване:

1. Миенето между стъпките е недостатъчно.
2. Тъканта е оставена да изсъхне с реактива върху нея.
3. Сгъване на тъканта и задържане на реактивите
4. Недостатъчно блокиране с Rabbit-to-Rabbit блок.
5. Тъканите съдържат ендогенен биотин.
6. Антигенът мигрира в тъканта.
7. Излишно количество на адхезив върху стъклото.
8. Недостатъчно блокиране със Супер блок.

Слабо оцветяване:

1. Концентрацията на първото антитяло е твърде ниска или времето за инкубиране е твърде кратко.
2. Реактивите са с изтекъл срок на годност.
3. Недостатъчно отстраняване на миещия буфер между стъпките, което води до разреждане на реактивите.
4. Контраоцветителя или средата за покриване са неподходящи и разтварят продукта на хромогенната реакция.
5. Температурата в помещението е прекалено ниска.
6. Първичното антитяло не разпознава антигена, който оцелява при фиксация и включване.
7. Излишна инкубация със Супер Блок.

Отсъствие на оцветяване:

1. Стъпките са оставени без контрол.
2. Отсъствие на антиген в тъканта.
3. Първичното антитяло не е с произход от мишка, плъх, заек или морско свинче.
4. Хромогена не е предназначен за използване с ензима, използван в процедурата (пероксидаза или алкална-фосфатаза).
5. Един или повече компоненти на кита са били инактивирани посредством загряване или неблагоприятни състояния

Превод от английски език

Antibodies-Online

Bone Morphogenetic Protein 15 (BMP15) antibody

Антиген:	Bone Morphogenetic Protein 15 (BMP15)
Синоними:	ODG2, POF4, GDF9B, Bmp-15, C86824, C87336, GDF-9B, AU015375, AU018861, AU021453, Gdf-9b, bmp-15, BMP-15, fi35a05, wu:fi35a05, BMP15
Реактивност:	Човек, Мишка, Плъх, Пиле, Куче, Свиня, Крава
Гостоприемник:	Заек
Клоналност:	Поликлонално
Конюгат:	Неконюгирано
Приложение:	Имуноблот (WB), ELISA, Имунохистохимия (Парафинови срези) (IHC (p)), Имунофлуоресценция IHC-P (IF (IHC-P))
Каталожен номер:	ABIN718841
Количество:	100 µl
Наличност:	.....

Информация за продукта

Имуноген:	KLH конюгиран синтетичен пептид получен от човешки BMP15
Изотип:	IgG
Кръстосана реактивност:	Плъх, Пиле, Куче, Свиня, Крава
Пречистване:	пречистен с Protein A колона и пептидна афинитетна хроматография
Общи данни:	----------
Молекулна маса:	45kDa

Детайли на приложение

Бележки за прилагане:	WB (1:100-500), ELISA (1:500-1000), IHC-P (1:100-500), IF (IHC-P) (1:100-500)
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Течен
Концентрация:	1 µg/µL
Буфер:	Буфериран разтвор, съдържащ 100 µg/mL BSA, 50 % глицерол и 0.09 % натриев азид
Консерванти:	натриев азид
Съхранение:	-20 °C
Срок на годност:	12 месеца при -20 °C

Поз.7-9-2
Превод от английски език

Лого на ScyTek Laboratories, Inc.

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

Rabbit-To-Rabbit Blocking Reagent

(Блокиращ Реактив за използване на заешки антитела върху заешка тъкан)

Описание на продукта: Rabbit-to-Rabbit реактивът на СкайТек е формулирана да осигури на изследователят система за оцветяване, способна да визуализира заешки антитела върху заешка тъкан. В повечето случаи 30-минутна инкубация с Rabbit-to-Rabbit блока ще елиминира фоновото оцветяване което се причинява от ендогенни имуноглобулини. За оптимални резултати, силно препоръчваме този реактив да се използва в комбинация със УлтраТек Анти-Заешка оцветяваща система на СкайТек

Произход: Коза
Антигенна специфичност: Анти-заешко
Ензимен конюгат: Няма
Хромоген субстрат: Няма

Процедура:

1. Депарафинирайте и рехидратирайте тъканните срези.
2. Ако е необходимо да намалите неспецифичния фон поради наличието на ендогенна пероксидаза,инкубирайте стъклата във водороден прекис за 10-15 мин.
3. Измийте двукратно в буфер.
4. Ако е необходимо, инкубирайте тъканите с ензим.
5. Измийте четирикратно в буфер.
6. Използвайте Супер Блок (СкайТек кат.№ AAA) и инкубирайте за 5 мин на стайна температура за блокиране на неспецифичното фоново оцветяване. **Забележка:** Не превишавайте повече от 10 мин. за да не се намали интензитета на реакцията.
7. Измийте еднократно в буфер.
8. Използвайте Rabbit-to-Rabbit блок и инкубирайте 10-60 мин. Времето за инкубация зависи от количеството на ендогенни имуноглобулини в тъканта.
9. Измийте четирикратно в буфер.
10. Използвайте първично антитяло и инкубирайте съгласно протокола на производителя.
11. Измийте четирикратно в буфер.
12. Използвайте УлтраТек Анти-Поливалент (СкайТек кат.№ ABN) и инкубирайте за 10-20 мин. на стайна температура.
13. Измийте четирикратно в буфер.
14. Използвайте УлтраТек HRP (СкайТек кат.№ ABL) или УлтраТек Алк-Фос (СкайТек кат.№ ABM) и инкубирайте за 10-20 мин. на стайна температура.
15. Измийте четирикратно в буфер.
16. Използвайте подходящ хромоген
17. Контраоцветете и покрийте.

Ръководство за оттраняване на неизправности

Преоцветяване:

1. Концентрацията на първото антитяло е много висока или времето за инкубиране е твърде продължителна.
2. Температурата по време на инкубиране е твърде висока.
3. Времето за инкубиране със свързващото антитяло или стрептавидин-пероксидазата е твърде продължителна.

Неспецифично фоново оцветяване:

1. Миенето между стъпките е недостатъчно.
2. Тъканта е оставена да изсъхне с реактива върху нея.
3. Сгъване на тъканта и задържане на реактивите
4. Недостатъчно блокиране с Rabbit-to-Rabbit блок.
5. Тъканите съдържат ендогенен биотин.
6. Антигенът мигрира в тъканта.
7. Излишно количество на адхезив върху стъклото.
8. Недостатъчно блокиране със Супер блок.

Слабо оцветяване:

1. Концентрацията на първото антитяло е твърде ниска или времето за инкубиране е твърде кратко.
2. Реактивите са с изтекъл срок на годност.
3. Недостатъчно отстраняване на миещия буфер между стъпките, което води до разреждане на реактивите.
4. Контраоцветителя или средата за покриване са неподходящи и разтварят продукта на хромогенната реакция.
5. Температурата в помещението е прекалено ниска.
6. Първичното антитяло не разпознава антигена, който оцелява при фиксация и включване.
7. Излишна инкубация със Супер Блок.

Отсъствие на оцветяване:

1. Стъпките са оставени без контрол.
2. Отсъствие на антиген в тъканта.
3. Първичното антитяло не е с произход от мишка, плъх, заек или морско свинче.
4. Хромогена не е предназначен за използване с ензима, използван в процедурата (пероксидаза или алкална-фосфатаза).
5. Един или повече компоненти на кита са били инактивирани посредством загряване или неблагоприятни състояния

Поз.7.10.

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

Ki-67 (M-19): sc-7846

Обща информация: ---

Хромозомна локализация: Генетичен локус: Mki67(мишка) – 7 F3

Източник: Ki-67 (M-19) е афинитетно пречистено козе поликлонално антитяло, получено срещу пептиден участък в С-терминалния край на Ki-67 от миши произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

За провеждане на конкурентни изследвание е наличен блокиращ пептид, sc-7846-P (100 µg пептид в 0.5 мл. PBS, съдържащ <0.1% натриев азид и 0.2% BSA)

Наличен е като phycoerythrin (sc-7846 PE), PerCP (sc-7846 PerCP) or PerCP-Cy5.5 (sc-7846 PCPC5) конюгати за флоуцитометрия, 100 теста; като fluorescein (sc-7846 FITC) или rhodamine (sc-7846 TRITC) конюгати за имунофлуоресценция, 200µg/ml; като Alexa Fluor® 405 (sc-7846 AF405), Alexa Fluor® 488 (sc-7846 AF488) или Alexa Fluor® 647 (sc-7846 AF647) конюгати за имунофлуоресценция; 100 µg/2 ml.

Alexa Fluor® е запазена марка на Molecular Probes, Inc., Oregon, USA

Приложения: Ki-67 (M-19) се препоръчва за доказване на Ki-67 от миши, плъши и в по-малка степен човешки произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500), флоуцитометрия (1 µg на 1 x 10^6 клетки) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Подходящо за използване като контролно антитяло за Ki-67 siRNA (h): sc-37613, Ki-67 siRNA (m): sc-37614, Ki-67 shRNA Plasmid (h): sc-37613-SH, Ki-67 shRNA Plasmid (m): sc-37614-SH, Ki-67 shRNA (h) Lentiviral Particles: sc-37613-V and Ki-67

shRNA (m) Lentiviral Particles: sc-37614-V.

Молекулна маса на Ki-67: 395/345 kDa.

Положителна контрола: MCF7 ядрен екстракт: sc-2149, Raji тотален клетъчен лизат или K-562 тотален клетъчен лизат: sc-2203.

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

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ORIGINAL ARTICLE

Stimulation of oval cell and hepatocyte proliferation by exogenous bombesin and neurotensin in partially hepatectomized rats

Stelios F Assimakopoulos, Athanassios C Tsamandas, Ilias H Alexandris, Christos Georgiou, Constantine E Vagianos, Chrisoula D Scopa

Stelios F Assimakopoulos, Department of Internal Medicine, University Hospital of Patras, Patras 26504, Greece
Athanassios C Tsamandas, Chrisoula D Scopa, Department of Pathology, University Hospital of Patras, Patras 26504, Greece
Ilias H Alexandris, Department of Surgery, General Hospital of Amfissa, Amfissa 33100, Greece
Christos Georgiou, Department of Biology, Section of Genetics, Cell Biology and Development, University of Patras, Patras 26100, Greece
Constantine E Vagianos, 1st Department of Surgical, "Saint Panteleimon" General Hospital of Nikaia, Piraeus 18454, Greece
Author contributions: Assimakopoulos SF, Vagianos CE and Scopa CD contributed to the conception and design; Alexandris IH, Georgiou C and Tsamandas AC contributed to the acquisition of data; Tsamandas AC and Assimakopoulos SF analyzed and interrelated the data; Assimakopoulos SF drafted the manuscript; Vagianos CE and Scopa CD critically revised the manuscript; all the authors have read and approved the final version of the manuscript.
Correspondence to: Stelios F Assimakopoulos, MD, PhD, Department of Internal Medicine, University Hospital of Patras, Patras 26504, Greece. sassim@upatras.gr
Telephone: +30-2610-999583 Fax: +30-2610-993982
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Abstract

AIM: To investigate the effect of the neuropeptides bombesin (BBS) and neurotensin (NT) on oval cell proliferation in partially hepatectomized rats not pretreated with a known hepatocyte inhibitor.

METHODS: Seventy male Wistar rats were randomly divided into five groups: I = controls, II = sham operated, III = partial hepatectomy 70% (PHx), IV = PHx + BBS (30 μg/kg per day), V = PHx + NT (300 μg/kg per day). Forty eight hours after liver resection, portal endotoxin levels and hepatic glutathione redox state were determined. α-fetoprotein (AFP) mRNA (*in situ* hybridisation), cytokeratin-19 and Ki67 antigen expression (immunohistochemistry) and apoptosis (TUNEL) were evaluated on liver tissue samples. Cells with morphological features of oval cells that were cytokeratin-19 (+) and AFP mRNA (+) were scored in morphometric analysis and their proliferation was recorded. In addition, the proliferation and apoptotic rates of hepatocytes were determined.

RESULTS: In the control and sham operated groups, oval cells were significantly less compared to groups III, IV and V ($P < 0.001$). The neuropeptides BBS and NT significantly increased the proliferation of oval cells compared to group III ($P < 0.001$). In addition, BBS and NT induced a significant increase of hepatocyte proliferation ($P < 0.001$), whereas it decreased their apoptotic activity ($P < 0.001$) compared to group III. BBS and NT significantly decreased portal endotoxemia ($P < 0.001$) and increased the hepatic GSH: GSSG ratio ($P < 0.05$ and $P < 0.001$, respectively) compared to group III.

CONCLUSION: BBS and NT stimulated oval cell proliferation in a model of liver regeneration, without use of concomitant suppression of hepatocyte proliferation as oval cell activation stimuli, and improved the hepatocyte regenerative response. This peptides-induced combined stimulation of oval cell and hepatocyte proliferation might serve as a possible treatment modality for several liver diseases.

Key words: Liver regeneration; Partial hepatectomy; Hepatic progenitor cells; Oval cells; Apoptosis; Proliferation; Oxidative stress

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INTRODUCTION

The efficiency of the regenerative response of human liver is of major clinical importance for patients' outcome in a number of diverse clinical conditions. When liver damage occurs, it is always followed by regeneration of the organ, which is mainly mediated by proliferation of the non-damaged mature hepatocytes[1,2]. When proliferation of the mature hepatocytes is suppressed, the majority of regeneration is carried out by oval cells, which have the capacity to differentiate into biliary epithelial cells or hepatocytes replacing the lost liver parenchyma[2-5]. Rat oval cells are frequently referred to as equivalent to hepatic progenitor cells in humans[3].

The hepatocyte is the most efÀcient cell for liver repopulation after injury; however, oval cells participate, possibly as an amplifying transit compartment for hepatocyte differentiation, in processes in which hepatocytes do not respond quickly enough or are unable to respond to proliferative stimuli[6]. Therefore, improving the efÀciency of the regenerative response of liver progenitor cells might have a substantial clinical impact, especially in cases of coexisting inhibition of mature hepatocyte proliferation, such as in viral hepatitis[7], chemical toxicity[8] and obstructive cholestasis[9]. For therapeutic application, a non-toxic activation of this stem cell compartment would have been required. Up until now, most experimental trials of pharmaceutical expansion of oval cell compartment have been conducted in models of mature hepatocyte proliferation inhibition[10-12]. Recent studies by our group have demonstrated that oval cells may also proliferate in a model of experimental liver cirrhosis, even in the absence of pretreatment with a known hepatocyte inhibitor[13].

Bombesin (BBS) and neurotensin (NT) are neuropeptides with a wide spectrum of actions on the gut-liver axis, influencing bile acid secretion, enterohepatic circulation, intestinal motility, blood Áow, secretion, nutrient absorption and immune response[14-19]. These agents activate diverse intracellular signals in hepatocytes, including induction of mitogenic, antioxidant and metabolic responses[20-23], and confer protection against liver injury and oxidative stress[21,22,24,25]. In our previous studies, we have shown that these neuropeptides reduce hepatic oxidative stress after partial hepatectomy (PHx) and improve the regenerative response of the cholestatic liver in rats[24,26].

This study was undertaken to investigate the possible effectiveness of BBS and NT as a pharmacological intervention for induction of oval cell proliferation in a widely applied experimental model of liver regeneration (PHx) without pretreatment with a known hepatocyte inhibitor.

MATERIALS AND METHODS

Animals

Seventy male albino Wistar rats, weighing 250-320 g, were used in the study. They were housed in stainless-steel cages, three rats per cage, under controlled temperature (23°C) and humidity conditions, with 12 h dark/ light cycles, and maintained on standard laboratory diet with tap water ad libitum throughout the experiment, except for an overnight fast before surgery.

The experiments were carried out according to international standards on animal welfare (86/ 609/ EEC) and to the guidelines of the Ethics Committee of Patras University Hospital. The study was approved by the local ethics committee.

Experimental design

Animals were divided randomly into Àve groups: group I (n = 10): non-operated controls; group II (n = 15): sham operated; group III (n = 15): PHx (70%); group IV (n = 15): PHx and BBS administration; group V (n = 15): PHx and NT administration.

Starting on day 0, the animals of groups IV and V were treated daily with BBS (10 μg/ kg, subcutaneously, three times a day) and NT (300 μg/ kg, intraperitoneally, once a day) respectively, while the animals of groups I, II and III were divided to receive daily either three subcutaneous or one intraperitoneal injection of 0.5 mL normal saline. Previous studies have shown that the route of saline administration does not affect the results[24]. On the 8th day, animals from groups III, IV and V underwent laparotomy and PHx (almost 70%) as described by Higgins and Andersson[27], while animals in group II underwent laparotomy and mobilization of the liver. The abdominal incision was closed in two layers with chromic 4-0 cat gut and 4-0 silk. All surgical procedures were performed under strict sterile conditions, using light ether anesthesia. Administration of BBS, NT and normal saline was continued for 48 h after surgery. On the 10th day, all animals were operated (group I) or reoperated on (groups II, III, IV and V), again under strict sterile conditions. Samples were obtained according to the experimental protocol, after which the rats were sacriÀced by exsanguination.

Peptides preparation

A stock solution of BBS (Sigma Chemical Co., St. Louis, Missouri, United States) was prepared by first dissolving the amount of peptide needed for the study in 1 mL sterile water containing 0.1% (w/ v) bovine serum albumin and then diluted with normal saline containing 1%

(w/ v) bovine serum albumin to a concentration of 3.5 μg BBS/ 0.1 mL. This solution was divided into equal aliquots of 0.1 mL that were stored in plastic tubes at -20°C. At the time of administration, a volume corresponding to a dose of 10 μg BBS/ kg body weight was taken from each aliquot and was further diluted with sterile saline to a Ànal volume of 0.5 mL that was injected subcutaneously three times daily. Selection of dose and route of administration was based on previous reports[24].

A stock solution of NT (Sigma Chemical Co., St. Louis, Missouri, United States) was prepared by Àrst dissolving the amount of peptide needed for the study in 1 mL sterile water containing 0.1% (w/ v) bovine serum albumin and then diluted with normal saline containing 0.1% (w/ v) bovine serum albumin to a concentration of 100 μg NT/ 0.1 mL. This solution was divided into equal aliquots of 0.1 mL that were stored in glass vials at -20°C. At the time of administration, a volume corresponding to a dose of 300 μg NT/ kg body weight was taken from each aliquot and was further diluted with sterile saline to a Ànal volume of 0.5 mL that was injected intraperitoneally once daily. Selection of dose and route of administration was based on previous reports[24].

Portal endotoxin measurements

For the determination of endotoxin concentrations in the portal vein, a laparotomy was performed in all groups, the portal vein was punctured and samples of 1 mL of blood were obtained. Endotoxin concentration was determined by the Limulus Amebocyte Lysate test (LAL, QCL-1000, Lonza, Walkersville, MD, United States) according to the manufacturer's instructions.

Determination of glutathione redox state

After laparotomy, a tissue sample of the liver of each animal was excised, washed in 9 g/ L of NaCl and homogenized in sodium phosphate buffer 10 mmol/ L, pH = 7.2 (containing 1 mmol/ L ethylenediaminetetraacetic acid and 1 mmol/ L butylated hydroxyanisole in 0.15% ethanol) by liquid nitrogen for the determination of glutathione redox state. Reduced glutathione was determined spectrophotometrically using Elman's reagent (DTNB) and oxidized glutathione (GSSG) was quantitated by a standard enzymic assay, as described previously[24].

Pathological analysis

In situ hybridization for α-fetoprotein expression in paraffin sections: For the detection of α-fetoprotein (AFP) mRNA (oval cell phenotype), a standard non-radioactive in situ hybridization method (ISH) was performed on paraffin sections, as described elsewhere[13]. The Hybridization/ Detection Complete System (MBI, Rockville, MD) and the digoxigenin (DIG)-labeled riboprobe for AFP subunit-1 in a 10-fold dilution in hybridization solution were used. Paraffin sections of embryonic rat liver tissue were used as a positive control. To conÀrm that the positive stain was speciÀc, the slides were processed in an identical way and hybridized with probes known to be complementary to sequences in the test sections (rat genomic DNA probes) (positive control probes). These probes (biotynilated oligonucleotide probes) were similar in length and GC content to AFP probe. For negative control purposes, the slides were processed in the same way but hybridized with heterologous probes. The latter were not complementary to any sequence in the test tissues. These negative control probes were similar in length and GC content to AFP probe.

Immunohistochemistry for the detection of CK19 and Ki67 proteins in paraffin sections: The detection of CK19 protein expression (oval cell phenotype)[5,13] and Ki67 expression (proliferation marker) relied on immunohistochemistry based on a streptavidin biotin peroxidase method (ImmunoCruz™ Staining systems sc-2053; Santa Cruz Biotechnology, Santa Cruz, CA). BrieÀy, 4-μm thick sections were dewaxed in xylene and hydrated through graded concentrations of alcohol. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 15 min. Sections were then processed in a microwave oven twice for 5 min each time at high power, and subsequently stained with anti-CK19 [goat polyclonal (sc-33119) (Santa Cruz Biotechnology, Santa Cruz, CA) in a dilution of 1:150] and anti-Ki67 [goat polyclonal antibody (M-19)] (sc-7846) (Santa Cruz Biotechnology, Santa Cruz, CA) in a dilution of 1:100]. All incubations were performed for 30 min at room temperature. Between the steps, sections were washed in TBS. Diaminobenzidine (Sigma Fast DAB tablets-D-4293, St Louis, MO) was used as the chromogen. Cytoplasmic staining for CK19 and nuclear staining for Ki67 were considered as positive. For negative control purposes, the same streptavidin-biotin technique was used on tissue sections in which 1% BSA in PBS was substituted for the primary antibody.

In situ labeling of fragmented DNA for the detection of apoptotic cells: On parafÀn sections, a standard terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick-end labeling (TUNEL) method was employed to detect the fragmented nuclear DNA associated with apoptosis[9]. For this purpose, the In Situ Cell Death Detection Kit, POD (Roche, United States) was used according to the manufacturer's instructions. After standard deparaffinization, hydration, incubation with proteinase K and blocking of endogenous peroxidase, tissue sections were incubated: (1) with TdT and DIG-dUTP (TUNEL reaction mixture) at 37°C for 60 min; and (2) with peroxidase converter anti-fluorescein antibody at 37°C for 30 min. Diaminobenzidine (Sigma Fast DAB tablets, D-4293, Sigma St. Louis, MO, United States) was used as the chromogen. For physiological positive controls, sections of rat small intestine were subjected to the same procedure. For negative controls, some slides were incubated with label solution that did not contain TdT.

Morphometric analysis: (1) Oval cell measurement: morphometric analysis for the evaluation of oval cell

Table 1 Portal Endotoxin concentrations and hepatic glutathione redox state (mean ± SD)

Markers	Control (I) (n = 10)	Sham (II) (n = 15)	PHx (III) (n = 15)	PHx + BBS (IV) (n = 15)	PHx + NT (V) (n = 15)
Endotoxin (EU/mL)	0.41 ± 0.07	0.40 ± 0.10	2.45 ± 0.62[a]	1.36 ± 0.53[b]	1.59 ± 0.44[b]
GSH: GSSG	11.55 ± 3.40	12.02 ± 2.85	20.43 ± 4.64[a]	25.88 ± 5.27[c]	28.69 ± 5.94[b]

[a]$P < 0.001$ *vs* sham; [b]$P < 0.001$ *vs* partial hepatectomy (PHx); [c]$P < 0.05$ *vs* PHx. BBS: Bombesin; NT: Neurotensin. GSH: Reduced glutathione hormone; GSSG: Oxidized glutathione.

presence was performed as described previously[13]. Briefly, sections were screened at low power and areas with increased oval cell staining were determined. Cells were scored when they fulÀlled the morphological criteria for oval cells (small cells with ovoid nuclei and scant cytoplasm) and expressed AFP mRNA, cytoplasmic protein CK19 and/ or nuclear protein Ki67. Cell counts were performed manually at a □ 400 magniÀcation (high power Àeld, HPF) using a 10 □ 10-microscope grid. The number of oval cells was determined by visual inspection of Àve non-overlapping different Àelds per section. The variance in oval cell counts from section to section in the same biopsy was < 10%. The average of these scores was then taken; and (2) Evaluation of proliferation and apoptosis in hepatocytes: immunohistochemical results regarding Ki67 expression and ISH results (TUNEL+ cells) were estimated for hepatocytes. All $Ki67^+$ hepatocytes were considered proliferating cells. Regarding apoptosis, in order to avoid its overestimation by the TUNEL method, hepatocytes were considered apoptotic only if, in addition to positive TUNEL stain, they displayed the morphological features of apoptosis on light microscopy (cytoplasmic fragmentation and nuclear condensation) and were not "surrounded" by inflammatory elements. Estimation of proliferation and apoptosis in each case was performed by a stereological method. Specifically, sections from each liver biopsy were viewed through a light microscope with □ 40 Àat Àeld objective. A square lattice of 100 points with a total surface area of 0.064 mm^2 was superimposed onto the tissue. Data were collected from a series of randomly selected 15 adjacent Àelds extending throughout the biopsy. For each Àeld a percentage value for each parameter ($Ki67^+$ cells, apoptotic cells) was obtained by dividing the points falling on stained tissue by the total number of measured points. Also, for each Àeld, the ratio of the obtained values (% $Ki67^+$ cells/ % apoptotic cells) was calculated as a balancing index expressing net cell turnover. It should be noted that the variance in cell counts from Àeld to Àeld in the same section was < 10%. The average of these scores was then taken and expressed as proliferation and apoptotic indexes and proliferation/ apoptosis ratio respectively for each case.

Statistical analysis

Data were analyzed using the SPSS statistical package (SPSS Inc., 2001, Release 11.0.0, United States). In groups I, II and III, data obtained from subcutaneously and intraperitoneally saline-treated rats were pooled, as there was no significant difference between differentially injected animals for all parameters studied. Results are expressed as mean (SD). Comparisons among multiple groups were performed using the one-way ANOVA, followed by Bonferroni's post hoc test when variances across groups were equal or by Dunnette's T3 post hoc test when variances were not equal. Variance equality was tested by Levene statistical analysis. In all cases, differences were considered signiÀcant when $P < 0.05$.

RESULTS

Portal endotoxin concentration

Hepatectomized animals (group III) presented significantly elevated endotoxin concentrations in portal blood compared with groups I and II ($P < 0.001$). Treatment with BBS or NT led to signiÀcantly lower endotoxin values in portal vein in groups IV and V ($P < 0.001$ vs group III, respectively) (Table 1).

Hepatic glutathione redox state

Evaluation of glutathione redox state showed significantly increased levels of reduced glutathione hormone (GSH):GSSG in hepatectomized rats of group III ($P < 0.001$ vs groups I and II). Administration of BBS or NT resulted in further increase of GSH:GSSG ratio in groups IV and V ($P < 0.05$ and $P < 0.001$ vs group III, respectively) (Table 1).

Oval cell detection and proliferation

Oval cells were present in all specimens studied. In the control and sham operated groups, oval cells were signiÀcantly less compared to groups III, IV and V ($P < 0.001$, Table 2). In PHx rats (group III) they were located in periportal areas and the formation of small ducts was occasionally recorded. Oval cells expressed CK19 (Figure 1A and B), AFP mRNA (Figure 1C and D) and Ki67. When rats subjected to PHx were treated with either BBS (group IV) or NT (group V), the levels of expression of all three molecules were signiÀcantly increased compared to group III ($P < 0.001$, Table 2).

Proliferation and apoptosis detection in hepatocytes

The proliferation index of hepatocytes was signiÀcantly higher in group III as compared to groups I and II ($P < 0.001$, respectively). Administration of BBS or NT in PHx rats induced a significant increase of hepatocyte proliferation in groups IV and V compared to group III ($P < 0.001$, respectively, Table 3) (Figure 2A and B).

Table 2 Morphometric analysis of oval cell presence and proliferation (marker-positive oval cells per high power field) (mean ± SD)

Markers	Control (I) (n = 10)	Sham (II) (n = 15)	PHx (III) (n = 15)	PHx + BBS (IV) (n = 15)	PHx + NT (V) (n = 15)
CK19 protein	1.65 ± 0.31	1.63 ± 0.28	6.22 ± 0.82[a]	18.51 ± 2.31[b]	19.37 ± 3.48[b]
AFP mRNA	1.34 ± 0.25	1.31 ± 0.21	5.45 ± 0.91[a]	16.32 ± 1.81[b]	17.53 ± 4.12[b]
Ki67 protein	1.28 ± 0.14	1.26 ± 0.11	5.22 ± 0.11[a]	15.61 ± 2.54[b]	16.64 ± 3.59[b]

[a]$P < 0.001$ *vs* sham; [b]$P < 0.001$ *vs* partial hepatectomy (PHx). BBS: Bombesin; NT: Neurotensin; AFP: α-fetoprotein.

Table 3 Hepatocytes' proliferation and apoptosis (mean ± SD)

Markers	Control (I) (n = 10)	Sham (II) (n = 15)	PHx (III) (n = 15)	PHx + BBS (IV) (n = 15)	PHx + NT (V) (n = 15)
Proliferation index	5.95 ± 1.33	6.12 ± 1.64	18.32 ± 3.11[a]	27.25 ± 4.13[b]	25.62 ± 3.41[b]
Apoptotic index	0	0	19.31 ± 4.16[a]	9.24 ± 2.65[b]	8.97 ± 4.14[b]
Proliferation/apoptosis	-	-	0.94 ± 0.74	2.99 ± 1.55[b]	2.85 ± 0.82[b]

[a]$P < 0.001$ *vs* sham; [b]$P < 0.001$ *vs* partial hepatectomy (PHx). BBS: Bombesin; NT: Neurotensin.

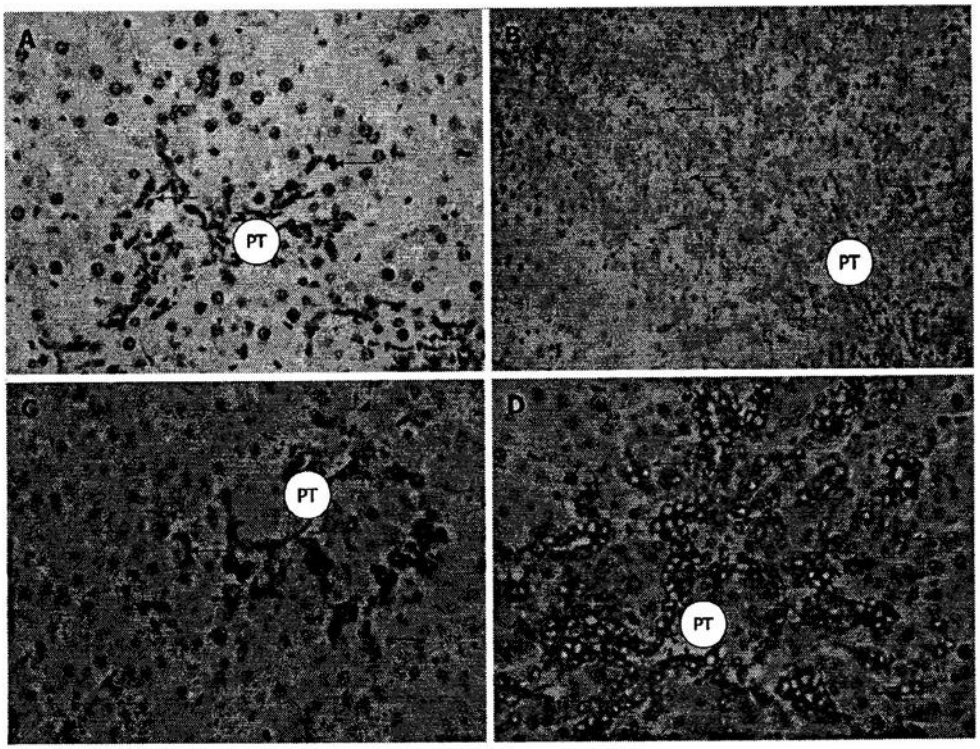

Figure 1 Microphotographs showing expression of CK19 from oval cells (arrows) in livers of group III (A) and group IV (B) and α-fetoprotein mRNA expression from oval cells (arrows) in group III (C) and group IV (D). In group IV there is higher oval cell presence as demonstrated by both markers [CK19: streptavidin biotin peroxidase (A) × 250, (B) × 100, α-fetoprotein mRNA: in situ hybridization × 250]. PT: Portal tract.

In control and sham operated rats, no apoptotic bodies were detected in hepatocytes. After PHx (group III), increased apoptotic activity was detected in lobules, whilst administration of BBS or NT significantly decreased the apoptotic index ($P < 0.001$, for groups IV and V compared with group III, Table 3) (Figure 2C and D). The proliferation/ apoptosis ratio was significantly increased in groups IV and V compared to group III ($P < 0.001$, respectively).

DISCUSSION

Effective liver regeneration after extended liver resection or hepatocytic necrosis is of great clinical importance and several experimental studies have focused on the pharmacological augmentation of these process. Experi-

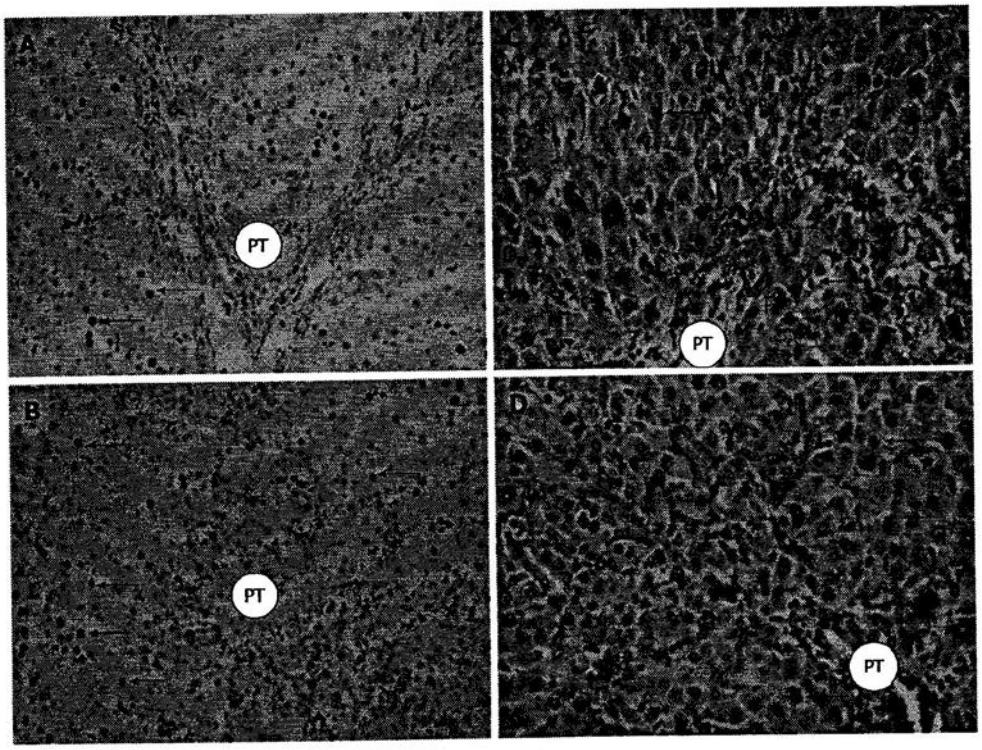

Figure 2 Microphotographs showing Ki67 (+) hepatocytes (arrows) from livers of group III (A) and group IV (B) and TUNEL (+) hepatocytes (arrows) in group III (C) and group IV (D). In group IV there are more Ki67 (+) hepatocytes (streptavidin biotin peroxidase × 100) and less TUNEL (+) hepatocytes (streptavidin biotin peroxidase and hematoxylin eosin × 250). PT: Portal tract.

mentally, the PHx and the carbon tetrachloride administration animal models have been widely used to simulate the clinical conditions of liver resection or hepatocytic necrosis, respectively. Previous studies have unequivocally shown that the hepatocytes are the replicating cells responsible for liver regeneration in these models and that progenitor cell activation leading to lineage generation is not observed during these processes[28]. Despite the low replicative rate of hepatocytes in the normal liver, these highly differentiated cells are not terminally differentiated and replicate in a highly regulated manner after loss of cell or tissue mass[28]. On the other hand, oval cells constitute a transit amplifying cell compartment, which is activated when hepatocyte proliferation is compromised[5,29]. The biological effect of several primary hepatocyte mitogens on hepatocytic proliferation has been extensively studied in experimental models but there are hardly any data about the influence of these compounds on oval cells[10,30,31]. Moreover, to the best of our knowledge, all in vivo pharmaceutical trials of oval cell compartment expansion have been conducted in conditions of mature hepatocyte proliferation inhibition, which does not allow safe conclusions on the clear effect of tested compounds on oval cell proliferation and also does not always simulate the clinical situation.

The present study evaluated the effect of the neuropeptides BBS and NT on oval cells and hepatocytes in partially hepatectomized rats, a widely applied model of liver regeneration. The experimental design did not include any treatment with known inhibitors of hepatocyte proliferation as a method for oval cell compartment activation. Therefore, we aimed at evaluating in vivo the potential net proliferative effect of tested peptides on oval cells for the first time. The results presented clearly demonstrate the proliferative effect of BBS and NT on oval cells in partially hepatectomized rats in conjunction with improvement of the regenerative response of mature hepatocytes evidenced by promotion of hepatocytes' proliferation and prevention of their apoptosis. Promotion of oval cell proliferation is of great clinical importance profoundly in cases of inhibition of hepatocyte proliferation as this cell type carries on the process of liver repopulation, as well as in cases of effective hepatocyte proliferation, providing a ready cell compartment to continue liver regeneration if a hepatocytic inhibitory insult arises. The presence of hepatic progenitor cells in failed livers at autopsy and in livers removed at transplantation indicates the importance of the efficiency of their regenerative response[32]. Agents that stimulate oval cell proliferation and differentiation, or transplantation of oval cells could be a potent therapeutic modality in the treatment of patients with fatal liver disease, such as fulminant hepatic failure.

In our previous studies with experimentally jaundiced rats, we have shown that BBS and NT attenuated oval cell proliferation in the cholestatic liver, inducing cell type-specific effects on oval cells, hepatocytes and cholangiocytes[26]. The findings of the present study with

promotion of oval cell proliferation suggest that oval cells' response to BBS and NT may be different depending on the type of liver injury. Taking into consideration our previous and current findings, one should assume that neuropeptides' effects on oval cells may be significant for the regeneration of a healthy liver (e.g. in living donor transplantation), but not relevant in an injured liver. However, in our previous experiments with bile duct ligated rats, BBS and NT signiÀcantly improved cholestatic liver injury, despite reduction of oval cell proliferation[26]. This Ànding might reÁect the fact that induction of hepatocytes' regenerative response by neuropeptides' action is effective for liver repair in cholestasis, diminishing the role of oval cell participation in this process. On the other hand, in the model of liver regeneration used in the present study, the induction of hepatocytes' regenerative response by BBS and NT might be relatively insufÀcient for an effective liver repopulation, thus activating the transit amplifying compartment of oval cells.

The biological effects of BBS and NT on hepatic oval cells of rats subjected to PHx may result from direct receptor-mediated action[33,34]. SpeciÀc G protein-coupled receptors of BBS and NT have been previously identiÀed in hepatocytes and cholangiocytes[33-35]. Similarly G protein-coupled receptors have been identiÀed in oval cells as well, and their expression has been interrelated with the activation with this stem cell compartment[36]. The growth pathways that govern activation and liver differentiation of liver stem cells after PHx are quite complex and not fully elucidated, involving interplay of diverse cytokines and growth factors such as the hepatocyte growth factor (HGF) and the transforming growth factor (TGF)-β[37]. Although a very rapid increase in tumor necrosis factor-α levels after hepatectomy (possibly endotoxin-induced) is considered as the Àrst step in activation of these growth factors[38], reduction of portal endotoxemia by BBS and NT, demonstrated in the present study, does not preclude the activation of oval cells via diverse HGF and TGF dependent pathways. In addition, an indirect mechanism of action of BBS and NT on oval cells in the regenerating liver could exist through the action of other gastrointestinal or systemic hormones released in response to these neuropeptides[39-44].

The present study also demonstrated that BBS and NT enhanced hepatocytes' regenerative response, attributed to increased proliferation and decreased apoptosis of mature hepatocytes and theoretically to increased transition of oval cells to mature hepatocytes. Estimation of hepatocytes' proliferation/ apoptosis ratio showed that BBS and NT induced a threefold net increase in proliferating over apoptotic hepatocytes in the regenerating liver. Hepatocytes' apoptosis, which is a major factor of a defective regenerative response, could have been attenuated by neuropeptides administration, either through a direct receptor-mediated mechanism or indirectly through reduction of hepatic oxidative stress and portal endotoxemia shown in the present study, with mechanisms previously reported[24,45]. According to the present and our previous results, liver regeneration takes place under low oxidative stress conditions; however, the further attenuation of hepatic oxidative stress induced by BBS and NT might contribute to the augmentation of the hepatocytes' regenerative response[24].

In conclusion, the present study demonstrates that the neuropeptides BBS and NT exert a net proliferative effect on oval cells in a model of liver regeneration without use of concomitant suppression of hepatocyte proliferation as oval cell activation stimuli. Concurrently, these factors promote hepatocyte proliferation and prevent its apoptosis, thus improving the hepatic regenerative response. Although the results from animal studies should be transferred with much caution in clinical practice, we feel that there is an emerging need for further evaluation of our findings, as the observed pharmacological combined stimulation of hepatocyte and oval cell proliferation might serve as a possible treatment modality for several liver diseases.

COMMENTS

Background

The regenerative response of human liver is of major clinical importance for patients' outcome in a number of diverse clinical conditions. The hepatocyte is the most ef¿cient cell for liver repopulation after injury; however, oval cells participate, possibly as an amplifying transit compartment for hepatocyte differentiation, in processes in which hepatocytes do not respond quickly enough or are unable to respond to proliferative stimuli.

Research frontiers

Pharmacological augmentation of the hepatic regenerative response in diverse types of liver injury has been the topic of intense research for several decades. Improving the ef¿ciency of the regenerative response of liver progenitor cells might have a substantial clinical impact, especially in cases of coexisting inhibition of mature hepatocyte proliferation, such as in viral hepatitis, chemical toxicity and obstructive cholestasis. Up until now, most experimental trials of pharmaceutical expansion of oval cell compartment have been conducted in animal models of mature hepatocyte proliferation inhibition by toxic chemical compounds. However, for therapeutic application a non-toxic activation of this stem cell compartment is required.

Innovations and breakthroughs

The present study evaluated the effect of the neuropeptides bombesin (BBS) and neurotensin (NT) on oval cells and hepatocytes in partially hepatectomized rats, a widely applied model of liver regeneration. The experimental design did not include any treatment with known inhibitors of hepatocyte proliferation as a method for oval cell compartment activation. Therefore, we aimed at evaluating in vivo the potential net proliferative effect of the tested peptides on oval cells for the first time. The results presented clearly demonstrate the proliferative effect of BBS and NT on oval cells in partially hepatectomized rats in conjunction with improvement of the regenerative response of mature hepatocytes, evidenced by promotion of hepatocytes' proliferation and prevention of their apoptosis.

Applications

Promotion of oval cell proliferation is of great clinical importance profoundly in cases of inhibition of hepatocyte proliferation as this cell type carries on the process of liver repopulation, as well as in cases of effective hepatocyte proliferation, providing a ready cell compartment to continue liver regeneration if a hepatocytic inhibitory insult arises. BBS and NT promoting oval cell and hepatocyte proliferation could be a potent therapeutic modality in the treatment of patients with several liver diseases, such as fulminant hepatic failure.

Peer review

This is an interesting observational study. The concept is new, the results are robust and may provide potential target for clinical patient care.

REFERENCES

1 **Evarts RP**, Hu Z, Omori N, Omori M, Marsden ER, Thorgeirsson SS. Precursor-product relationship between oval cells and hepatocytes: comparison between tritiated thymidine and bromodeoxyuridine as tracers. *Carcinogenesis* 1996; **17**: 2143-2151

2 **Pintilie DG**, Shupe TD, Oh SH, Salganik SV, Darwiche H, Petersen BE. Hepatic stellate cells' involvement in progenitor-mediated liver regeneration. *Lab Invest* 2010; **90**: 1199-1208

3 **Roskams TA**, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytiroglou P, Knisely AS, Kojiro M, Lefkowitch JH, Nakanuma Y, Olynyk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 2004; **39**: 1739-1745

4 **Kisseleva T**, Gigante E, Brenner DA. Recent advances in liver stem cell therapy. *Curr Opin Gastroenterol* 2010; **26**: 395-402

5 **Petersen BE**, Zajac VF, Michalopoulos GK. Hepatic oval cell activation in response to injury following chemically induced periportal or pericentral damage in rats. *Hepatology* 1998; **27**: 1030-1038

6 **Paku S**, Schnur J, Nagy P, Thorgeirsson SS. Origin and structural evolution of the early proliferating oval cells in rat liver. *Am J Pathol* 2001; **158**: 1313-1323

7 **Tsamandas AC**, Syrokosta I, Thomopoulos K, Zolota V, Dimitropoulou D, Liava A, Coupoulou AA, Siagris D, Petsas T, Karatza C, Gogos CA. Potential role of hepatic progenitor cells expression in cases of chronic hepatitis C and their relation to response to therapy: a clinicopathologic study. *Liver Int* 2006; **26**: 817-826

8 **Lemire JM**, Shiojiri N, Fausto N. Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. *Am J Pathol* 1991; **139**: 535-552

9 **Karavias DD**, Tsamandas AC, Tepetes K, Kritikos N, Kourelis T, Ravazoula P, Vagenas K, Siasos N, Mirra N, Bonikos DS. BCL-2 and BAX expression and cell proliferation, after partial hepatectomy with and without ischemia, on cholestatic liver in rats: an experimental study. *J Surg Res* 2003; **110**: 399-408

10 **László V**, Dezso K, Baghy K, Papp V, Kovalszky I, Sáfrány G, Thorgeirsson SS, Nagy P, Paku S. Triiodothyronine accelerates differentiation of rat liver progenitor cells into hepatocytes. *Histochem Cell Biol* 2008; **130**: 1005-1014

11 **Hasuike S**, Ido A, Uto H, Moriuchi A, Tahara Y, Numata M, Nagata K, Hori T, Hayashi K, Tsubouchi H. Hepatocyte growth factor accelerates the proliferation of hepatic oval cells and possibly promotes the differentiation in a 2-acetylaminofluorene/partial hepatectomy model in rats. *J Gastroenterol Hepatol* 2005; **20**: 1753-1761

12 **Piscaglia AC**, Shupe TD, Oh SH, Gasbarrini A, Petersen BE. Granulocyte-colony stimulating factor promotes liver repair and induces oval cell migration and proliferation in rats. *Gastroenterology* 2007; **133**: 619-631

13 **Tsamandas AC**, Antonacopoulou A, Kalogeropoulou C, Tsota I, Zabakis P, Giannopoulou E, Liava A, Tzelepi V, Tepetes K, Petsas T, Kardamakis D, Kalofonos HP. Oval cell proliferation in cirrhosis in rats. An experimental study. *Hepatol Res* 2007; **37**: 755-764

14 **Gyires K**. Neuropeptides and gastric mucosal homeostasis. *Curr Top Med Chem* 2004; **4**: 63-73

15 **Chu KU**, Higashide S, Evers BM, Ishizuka J, Townsend CM, Thompson JC. Bombesin stimulates mucosal growth in jejunal and ileal Thiry-Vella fistulas. *Ann Surg* 1995; **221**: 602-609; discussion 609-611

16 **Evers BM**, Izukura M, Townsend CM, Uchida T, Thompson JC. Differential effects of gut hormones on pancreatic and intestinal growth during administration of an elemental diet. *Ann Surg* 1990; **211**: 630-636; discussion 636-638

17 **Gui X**, Dobner PR, Carraway RE. Endogenous neurotensin facilitates enterohepatic bile acid circulation by enhancing intestinal uptake in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G1413-G1422

18 **Krco CJ**, Gores A, Go VL. Gastrointestinal regulatory peptides modulate mouse lymphocyte functions under serum-free conditions in vitro. *Immunol Invest* 1986; **15**: 103-111

19 **Uluutku AH**, Akin ML, Kurt Y, Yucel E, Cermik H, Avsar K, Celenk T. Bombesin in short bowel syndrome. *J Invest Surg* 2004; **17**: 135-141

20 **Hasegawa K**, Kar S, Carr BI. Stimulation of hepatocyte DNA synthesis by neurotensin. *J Cell Physiol* 1994; **158**: 215-222

21 **Li JY**, Wang L, Zhang XJ. [Cytoprotective effect of neurotensin on acetaminophen induced liver injury in relation to glutathione system]. *Sheng Li Xue Bao* 1994; **46**: 168-175

22 **Cascales M**, Martin-Sanz P, Bosca L. Phorbol esters, bombesin and insulin elicit differential responses on the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase system in primary cultures of foetal and adult rat hepatocytes. *Eur J Biochem* 1992; **207**: 391-397

23 **Esparza JL**, Gómez M, Romeu M, Mulero M, Sánchez DJ, Mallol J, Domingo JL. Aluminum-induced pro-oxidant effects in rats: protective role of exogenous melatonin. *J Pineal Res* 2003; **35**: 32-39

24 **Alexandris IH**, Assimakopoulos SF, Vagianos CE, Patsoukis N, Georgiou C, Nikolopoulou V, Scopa CD. Oxidative state in intestine and liver after partial hepatectomy in rats. Effect of bombesin and neurotensin. *Clin Biochem* 2004; **37**: 350-356

25 **Assimakopoulos SF**, Vagianos CE, Zervoudakis G, Filos KS, Georgiou C, Nikolopoulou V, Scopa CD. Gut regulatory peptides bombesin and neurotensin reduce hepatic oxidative stress and histological alterations in bile duct ligated rats. *Regul Pept* 2004; **120**: 185-193

26 **Assimakopoulos SF**, Tsamandas AC, Georgiou CD, Vagianos CE, Scopa CD. Bombesin and neurotensin exert antiproliferative effects on oval cells and augment the regenerative response of the cholestatic rat liver. *Peptides* 2010; **31**: 2294-2303

27 **Higgins GM**, Anderson RM. Experimental pathology of the liver.I. Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol* 1931; **12**: 186-202

28 **Fausto N**, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev* 2003; **120**: 117-130

29 **Sánchez A**, Factor VM, Schroeder IS, Nagy P, Thorgeirsson SS. Activation of NF-kappaB and STAT3 in rat oval cells during 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration. *Hepatology* 2004; **39**: 376-385

30 **Columbano A**, Shinozuka H. Liver regeneration versus direct hyperplasia. *FASEB J* 1996; **10**: 1118-1128

31 **Nishino M**, Iimuro Y, Ueki T, Hirano T, Fujimoto J. Hepatocyte growth factor improves survival after partial hepatectomy in cirrhotic rats suppressing apoptosis of hepatocytes. *Surgery* 2008; **144**: 374-384

32 **Fujita M**, Furukawa H, Hattori M, Todo S, Ishida Y, Nagashima K. Sequential observation of liver cell regeneration after massive hepatic necrosis in auxiliary partial orthotopic liver transplantation. *Mod Pathol* 2000; **13**: 152-157

33 **Junco M**, Díaz-Guerra MJ, Boscá L. Differential calcium mobilization by vasopressin, angiotensin II, gastrin-releasing peptide, and adenosine triphosphate in adult and fetal hepatocytes. Relevance for the activation of calcium-dependent enzymes. *Endocrinology* 1993; **132**: 309-318

34 **Méndez M**, Souazé F, Nagano M, Kelly PA, Rostène W, Forgez P. High affinity neurotensin receptor mRNA distribution in rat brain and peripheral tissues. Analysis by quantitative RT-PCR. *J Mol Neurosci* 1997; **9**: 93-102

35 **Cho WK**, Boyer JL. Characterization of ion transport mecha-

nisms involved in bombesin-stimulated biliary secretion in rat cholangiocytes. *J Hepatol* 1999; **30**: 1045-1051

36 **Sautin YY**, Jorgensen M, Petersen BE, Saulnier-Blache JS, Crawford JM, Svetlov SI. Hepatic oval (stem) cell expression of endothelial differentiation gene receptors for lysophosphatidic acid in mouse chronic liver injury. *J Hematother Stem Cell Res* 2002; **11**: 643-649

37 **Sánchez A**, Fabregat I. Growth factor- and cytokine-driven pathways governing liver stemness and differentiation. *World J Gastroenterol* 2010; **16**: 5148-5161

38 **Nagy P**, Kiss A, Schnur J, Thorgeirsson SS. Dexamethasone inhibits the proliferation of hepatocytes and oval cells but not bile duct cells in rat liver. *Hepatology* 1998; **28**: 423-429

39 **Jensen RT**, Battey JF, Spindel ER, Benya RV. International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. *Pharmacol Rev* 2008; **60**: 1-42

40 **Dolais-Kitabgi J**, Kitabgi P, Brazeau P, Freychet P. Effect of neurotensin on insulin, glucagon, and somatostatin release from isolated pancreatic islets. *Endocrinology* 1979; **105**: 256-260

41 **Walker JP**, Khalil T, Wiener I, Fagan CJ, Townsend CM, Greeley GH, Thompson JC. The role of neurotensin in human gallbladder motility. *Ann Surg* 1985; **201**: 678-683

42 **Eysselein VE**, Hesse WH, Eberlein GA, Koelbel C, Niebel W, Goebell H. Action of neurotensin on meal-stimulated gastric acid secretion in the dog. *Scand J Gastroenterol* 1990; **25**: 29-39

43 **Mazzocchi G**, Malendowicz LK, Rebuffat P, Gottardo G, Nussdorfer GG. Neurotensin stimulates CRH and ACTH release by rat adrenal medulla in vitro. *Neuropeptides* 1997; **31**: 8-11

44 **Printz H**, Göke B, Fehmann HC, Weiershausen S, Nustede R, Neurath M, Rothmund M. Partial hepatectomy affects pancreatic size and function in rats. *Pancreas* 1993; **8**: 233-239

45 **Yoshimoto N**, Togo S, Kubota T, Kamimukai N, Saito S, Nagano Y, Endo I, Sekido H, Nagashima Y, Shimada H. Role of transforming growth factor-beta1 (TGF-beta1) in endotoxin-induced hepatic failure after extensive hepatectomy in rats. *J Endotoxin Res* 2005; **11**: 33-39

S- Editor Wu X **L- Editor** Roemmele A **E- Editor** Zheng XM

Поз.7.11.

Превод от английски език

Лого на Abcam

Информационен лист

Анти- Aflatoxin антитяло **[ATB]**

Наименование на продукта	Анти- Aflatoxin антитяло [ATB]
Код на продукта	ab1985
Обем	200 µg
Описание	Мише моноклонално [ATB] срещу Aflatoxin
Специфичност	Това антитяло разпознава свободни афлатоксини B1 и B2. Не показва кръстосана реактивност с G1, G2 и M1
Тествани приложения	ELISA
Видова реактивност	Aspergillus flavus
Имуноген	Afiatoxin

Свойства

Форма	Течна
Инструкции за съхранение	Доставя се при +4°C. След доставка разфасовайте и съхранявайте при -20°C. Избягвайте повторно замразяване/размразяване.
Буфер за съхранение	Консервант: 0.1% натриев азид Съставки: PBS, pH 7.4
Концентрация	200 µg при 2 mg/ml
Чистота	Протеин G пречистване. Пречистването е тествано чрез електрофореза
Клоналност	Поликлонално
Подтип	IgG

Приложения

ELISA

Antibodies-Online

Caspase 3, Apoptosis-Related Cysteine Peptidase (CASP3) antibody

Антиген:	Caspase 3, Apoptosis-Related Cysteine Peptidase (CASP3)
Синоними:	xcpp32, Lice, CPP32, SCA-1, CPP32B, CC3, CG14902, Cas3, Casp 3, Casp3, Caspase-3, DECAY, Decay, Dmel\\CG14902, Drosophila executioner caspase related to Apopain/Yama, caspase 3, caspase-3, CG7788, DRICE, Dmel\\CG7788, DrICE, DrIce, Drice, ICE, crice, drICE, drIce, drice, ice
Епитоп:	Ser29, N-Term
Реактивност:	Човек
Гостоприемник:	Заек
Клоналност:	Моноклонално (Y83-77)
Конюгат:	Неконюгирано
Приложение:	Имуноблот (WB), ELISA, Имунохистохимия (Криостатни срези), Имунофлуоресценция (IF)
Каталожен номер:	ABIN189202
Количество:	100 µl
Наличност:	.....

Информация за продукта

Имуноген:	Синтетичен пептид кореспондиращ с остатъците след Ser29 на човешка caspase-3 (N-края на p17 субединица)
Клон:	Y83-77
Изотип:	IgG
Специфичност:	Това антитяло разпознава единствено активната форма на caspase-3 (p17 субединица). Реагира с човек и мишка. Не реагира с плъх. Не е тествано при други видове
Кръстосана реактивност:	човек и мишка
Пречистване:	Пречистен
Общи данни:	----------

Детайли на приложение

Бележки за прилагане:	Този продукт се използва за: Имуноблот, Имунохистохимия на парафинови срези, Имуноцитохимия. При Имуноблот, антитялото открива ивица с приблизителна маса от 17kDa. Препоръчвани разреждания: Имунохистохимия / Имунофлуоресценция 1:100-250, Имунохистохимия 1:10-1:500, Имунохистохимия на парафинови срези 1:250, Имуноблот 1:1000-10000
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Течен
Буфер:	PBS 49 % , Glycerol 50 % , BSA 0.05 % , Sodium Azide
Консерванти:	натриев азид
Съхранение:	-20 °C

Поз.7.13.
Превод от английски език

Antibodies-Online

Tubulin (TUB) antibody

Антиген:	Tubulin (TUB)
Реактивност:	Пиле
Гостоприемник:	Мишка
Клоналност:	Моноклонално (655)
Конюгат:	Неконюгирано
Приложение:	Имуноблот (WB), ELISA, Имунохистохимия (Криостатни срези), Имунофлуоресценция (IF)
Каталожен номер:	ABIN934110
Количество:	1 мл
Наличност:	.....

Информация за продукта

Имуноген:	Антитялото срещу тубулин е получено в мишка, използвайки клетъчен препарат от птича скелетна мускулатура
Клон:	655
Изотип:	IgM

Детайли на приложение

Бележки за прилагане:	Оптималните условия трябва да се определят от потребителя
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Течен
Буфер:	Доставя се в течна форма. Разредете незабавно преди употреба с PBS или TBS. Съдържа 0.1% натриев азид.
Консерванти:	натриев азид
Съхранение:	4 °C

Поз.7.14.
Превод от английски език

Antibodies-Online

Mucin 1, Cell Surface Associated (MUC1) (C-Term) antibody

Антиген:	Mucin 1, Cell Surface Associated (MUC1)
Синоними:	CA 15-3, CD227, EMA, H23AG, KL-6, MAM6, MCKD1, MUC-1, MUC-1/SEC, MUC-1/X, MUC1/ZD, PEM, PEMT, PUM, Muc-1, MUC1
Епитоп:	C-Term
Реактивност:	Заек, Мишка, Куче, Плъх, Свиня, Крава, Човек
Гостоприемник:	Заек
Клоналност:	Поликлонално
Конюгат:	Неконюгирано
Приложение:	Имуноблот (WB), Имунохистохимия
Каталожен номер:	ABIN310153
Количество:	50 µg
Наличност:	.....

Информация за продукта

Имуноген:	Синтетичен пептид отговарящ на карбоксилния край на човешки MUC1
Секвенция:	GQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSTDRSPYEK VSAGNGGSSL
Реактивност:	Морско свинче : 100 %, Кон : 100 %, Човек : 100 %, Мишка : 100 %, Свиня : 100 %, Заек : 100 %, Плъх : 100 %, Говедо : 90 %
Характеристики:	Това антитяло е заешко поликлонално срещу MUC1. То е валидирано за Имуноблот и Имунохистохимия
Пречистване:	Афинитетно пречистено
Общи данни:	----------
Молекулна маса:	22 kDa
Изследователска област	Тумор, Екстрацелуларен матрикс

Детайли на приложение

Бележки за прилагане:	Оптималните условия трябва да се определят от потребителя
Коментар:	Големина на антигена: 203 AK
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Лиофилизиран
Разтваряне:	Добавете 50 µL дестилирана вода
Концентрация:	1 mg/mL
Буфер:	PBS с 2 % сукроза
Съхранение:	-20 °C

Antibodies-Online

Bestrophin 1 (BEST1) (C-Term) antibody

Антиген:	Bestrophin 1 (BEST1)
Синоним:	CG6264, Dbest, Dmel\\CG6264, anon-WO0118547.380, best, dBest1, dbest1, dmBest1, BEST1, VMD2, best-1, ARB, BEST, BMD, RP50, TU15B, Bmd, Vmd2, mBest1
Епитоп:	C-Term
Реактивност:	Човек
Гостоприемник:	Мишка
Клоналност:	Моноклонално (E6-6)
Конюгат:	Неконюгирано
Приложение:	Имуноблот (WB), ELISA, Имуноцитохимия, Имунохистохимия на парафинови и криостатни срези, Имунофлуоресценция (IF), Имунопреципитация
Каталожен номер:	ABIN152509
Количество:	0.1 mL
Наличност:	.....

Информация за продукта

Имуноген:	Синтетичен пептид конюгиран към KLH и кореспондиращ с карбоксдилния край на човешки Bestrophin 1 (KDHMDPYWALENRDEAHS)
Клон:	E6-6
Изотип:	IgG kappa
Пречистване:	Асцитна течност

Детайли на приложение

Бележки за прилагане:	При Имуноблот антитялото разпознава ивица с приблизителна молекулна маса ~68 kDa. Подходящ е и за имунофлуоресценция и имунохистохимия на парафинови срези Препоръчвани разреждания: Имуноцитохимия/Имунофлуоресценция – в зависимост от метода; Имунохистохимия на парафинови и криостатни срези; Имунопреципитация, Имуноблот 1:1000
Протокол:	Протокол за Имуноблот: 1. Сепарирайте клетъчните лизати на SDS-PAGE 2. Трансферирайте протеините върху PVDF мембрана 3. Блокирайте мембраната със сухо обезмаслено мляко в PBS + 0.1 % Tween 20 (с 0.1mM CaCl2 и 1mM MgCl2) за 1 час на стайна температура 4. Разредете антитялото 1:1000 в 10 мл. Блокиращ буфер и инкубирайте за 1 час на стайна температура 5. Измийте мембраната с блокиращ буфер 3x 5-10 мин 6. Разредете второто антитяло в пресен блокиращ буфер и инкубирайте за 1 час на стайна температура 7. Измийте мембраната в блокиращ буфер 5x 8 мин. и промийте 1x с PBS (съдържщ 0.1mM CaCl2 и 1mM MgCl2). 8. Детектирайте комплекса протеин-антитяло с алкална фосфатаза (ако

ползвате NBT/BCIP) или с HRP (ако позвате ECL.

Подготовка на клетъчни лизати:

1. Лизатите се приготвят в лизиращ буфер [50mM Tris-HCl, pH 8 / 120mM NaCl / 0.5 % Nonidet P-40 / 10 µg/ml aprotinin / 10 µg/ml leupeptin / 1mM phenylmethylsulfonyl fluoride / 1mM sodium orthovanadate].

Ограничения: Само за изследователски цели

Манипулации

Формат: Течен

Консерванти: натриев азид

Съхранение: -20 °C. Може да се разфасова в аликвоти и да се съхранява на -20 °C или -80 °C

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Bestrophin 1 (BEST1) (C-Term) antibody

Details for Product No. ABIN152509

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Antigen	Bestrophin 1 (BEST1)
Synonyms	CG6264, Dbest, DmelI\CG6264, anon-WO0118547.380, best, dBest1, dbest1, dmBest1, BEST1, VMD2, best-1, ARB, BEST, BMD, RP50, TU15B, Bmd, Vmd2, mBest1
Epitope	Alternatives C-Term C-Term (5), Middle Region (4), N-Term (3), AA 1-604 (1), AA 31-52 (1), AA 361-460 (1), AA 445-464 (1), AA 605-614 (1), Internal Region (1)
Reactivity	Alternatives Human Human (15), Monkey (4), Mouse (Murine) (4), Rat (Murine) (3), Pig (Porcine) (1)
Host	Alternatives Mouse Rabbit (20), Mouse (6)
Clonality (Clone)	Monoclonal (68-6)
Conjugate	Alternatives Un-conjugated Biotin (3), FITC (3)
Application	Alternatives Western Blotting (WB), Immunocytochemistry (ICC), Immunofluorescence (IF), Immunohistochemistry (Frozen Sections) (IHC (fro)), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP) Western Blotting (WB) (17), Immunohistochemistry (IHC) (8), Immunofluorescence (IF) (6), ELISA (5), Immunoprecipitation (IP) (5), Immunohistochemistry (Frozen Sections) (IHC (fro)) (2), Immunoassay (IA) (1), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)) (1)
Publications	2 references available
Quantity	0.1 mL
Shipping to	United States (Change)

Catalog No. ABIN152509
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Product details

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Immunogen	Synthetic peptide conjugated to KLH corresponding to the C-terminus of human Bestrophin 1 (KDHMDPYWALENRDEAHS)
Clone	E6-6
Isotype	IgG1 kappa
Specificity	Bestrophin (E6-6)
No Cross-Reactivity	Rat (Rattus)
Cross-Reactivity (Details)	Other species not tested.
Purification	Ascites

Target details

References Images Handling Application Details back to top

Alternative Name	Bestrophin-1
Background	Best macular dystrophy (BMD) or vitelliform macular dystrophy (VMD2), is an autosomalform of macular degeneration, characterized by a depressed light peak in theelectrooculogram (EOG). It is inherited and has an early onset. Bestrophin is a 68 kDabasolateral plasma membrane protein encoded by the VMD2 gene. Bestrophin'sfunction is still unknown, but data suggests that it is a chloride channel that plays a rolein generating the altered EOG in Best disease patients. In addition, Bestrophin is ausefu biochemical and histological marker of RPE (retinal pigment epithelial cells) cells. Gene Symbol: BEST1
Gene ID	7439, 293735
UniProt	O76090

Application Details

References Images Handling Target details back to top

Application Notes	By Western blot, this antibody recognizes a band at ~68 kDa representing Bestrophin.** It is also useful for immunofluorescence. **Please see protocol for treatment of cell extract. Use in Immunohistochemistry-Paraffin reported in scientific literature (PMID 24345323) Recommended dilutions: Immunocytochemistry/Immunofluorescence Assay dependent, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Western Blot 1:1000
Protocol	Western Blot protocol specific for Bestrophin 1 Antibody Procedure Guide for NB 300-164 Monoclonal Anti-BestrophinWestern Blot Procedure 1. Run cell lysates** on an SDS-PAGE gel. . Transfer the proteins to PVDF. . Block the membrane in 1 % Carnation instant milk in PBS + 0.1 % Tween 20 (with 0.1mM CaCl2 and 1mM MgCl2) for 1 hour at RT. . Dilute the anti-Bestrophin [NB 300-164] to 1:1,000 in 10 mL of fresh blocking buffer and incubate for 1 hour at RT. . Wash the membrane with blocking buffer, 3x 5-10 minutes. . Dilute the secondary antibody in fresh blocking buffer, as recommended by the secondary vendor and incubate for 1hour at RT. . Wash the membrane with blocking buffer, 5x 8 minutes and rinse 1x with PBS (containing 0.1mM CaCl2 and 1mM MgCl2). . Detect the protein-antibody complex with alkaline phosphatase, if using NBT/BCIP or with HRP, if using ECL.**Cell Lysate PreparationA. Lysates were prepared in lysis buffer [50mM Tris-HCl, pH 8 / 120mM NaCl / 0.5 % Nonidet P-40 / 10 µg/ml aprotinin / 10 µg/ml leupeptin / 1mM phenylmethylsulfonyl fluoride / 1mM sodium orthovanadate].B. Total protein content was determined by bicinchoninic acid assay (Pierce).
Restrictions	For Research Use only

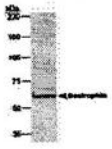
Handling

References Images Application Details Target details back to top

Format	Liquid
Preservative	Sodium azide
Precaution of Use	WARNING: Reagents contain sodium azide. Sodium azide is very toxic if ingested or inhaled. Avoid contact with skin, eyes, or clothing. Wear eye or face protection when handling. If skin or eye contact occurs, wash with copious amounts of water. If ingested or inhaled, contact a physician immediately. Sodium azide yields toxic hydrazoic acid under acidic conditions. Dilute azide-containing compounds in running water before discarding to avoid accumulation of potentially explosive deposits in lead or copper plumbing.
Handling Advice	Avoid freeze-thaw cycles
Storage	-20 °C
Storage Comment	Aliquot and store at -20 °C or -80 °C.

Images

References Handling Application Details Target details back to top



Western blot detection of Bestrophin (68 kDa) from human RPE cell lysate using ARIN152509 (1:1000).
Western blot detection of Bestrophin (68 kDa) from human RPE cell lysate using ABIN152509 (1:1000).

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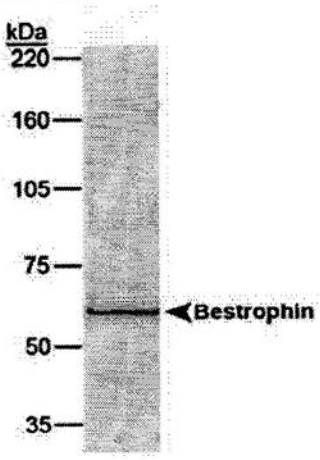

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Marmorstein, Marmorstein, Rayborn et al.: "Bestrophin, the product of the Best vitelliform macular dystrophy gene (VMD2), localizes to the basolateral plasma membrane of the retinal pigment epithelium." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 97, Issue 23, pp. 12758-63, 2000 (PubMed).

General Klimanskaya, Hipp, Rezai et al.: "Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics." in: **Cloning and stem cells**, Vol. 6, Issue 3, pp. 217-45, 2005 (PubMed).

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- BCL2/adenovirus E1B 19kDa Interacting Protein 3-Like (BNIP3L) Antibody
- BCL2/adenovirus E1B 19kDa Interacting Protein 3 (BNIP3) Antibody

- Bestrophin 2 (BEST2) Antibody
- Bestrophin 3 (BEST3) Antibody
- Bestrophin 4 (BEST4) Antibody
- beta 1,3-Galactosyltransferase-Like (B3GALTL) Antibody
- beta-1,3-Glucuronyltransferase 1 (Glucuronosyltransferase P) (B3GAT1) Antibody
- beta-1,3-Glucuronyltransferase 2 (Glucuronosyltransferase S) (B3GAT2) Antibody
- beta-1,3-Glucuronyltransferase 3 (Glucuronosyltransferase I) (B3GAT3) Antibody
- beta-1,3-N-Acetylgalactosaminyl Transferase 2 (B3GALNT2) Antibody
- beta-1,3-N-Acetylgalactosaminyltransferase 1 (Globoside Blood Group) (B3GALNT1) Antibody
- beta-1,4-N-Acetyl-Galactosaminyl Transferase 1 (B4GALNT1) Antibody

Checked performance

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

β-catenin (H-102): sc-7199

Обща информация: ----------

Хромозомна локализация: Генетичен локус: CTNNB1 (човек) - 3p22.1; Ctnnb1 (мишка) - 9 F4.

Източник: β-catenin (H-102) е заешко поликлонално антитяло, получено срещу аминокиселините 680-781 в карбоксилния край на β-catenin от човешки произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

Приложения: β-catenin (H-102) се препоръчва за доказване на β-catenin от миши, плъши, човешки, *Xenopus laevis* и zebrafish произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500), Имунохистохимия (включително парафинови срези) (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

β-catenin (H-102) се препоръчва за доказване на β-catenin в допълнителни видове, включително кон, куче, говедо, свиня и птица.

Подходящо за използване като контролно антитяло за β-catenin siRNA (h): sc-29209, β-catenin siRNA (m): sc-29210, β-catenin shRNA Plasmid (h): sc-29209-SH, β-catenin shRNA Plasmid (m): sc-29210-SH, β-catenin shRNA (h) Lentiviral Particles: sc-29209-V и β-catenin shRNA (m) Lentiviral Particles: sc-29210-V.

Молекулна маса на β-catenin: 92 kDa.

Положителна контрола: SK-BR-3 клетъчен лизат: sc-2218, HeLa тотален клетъчен лизат: sc-2200 или A-431 тотален клетъчен лизат: sc-2201.

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

p-ERK 1/2 (Thr 202/Tyr 204): sc-16982

Обща информация: ----------

Хромозомна локализация: Генетичен локус: MAPK3 (човек) - 16p11.2, MAPK1 (човек) - 22q11.21; Mapk3 (мишка) 7 F3, Mapk1 (мишка) 16 A3.

Източник: p-ERK 1/2 (Thr 202/Tyr 204) е налично като козе (sc-16982) или заешко (sc-16982-R) поликлонално афинитетно пречистено антитяло, получено срещу къса аминокиселинна секвенция, съдържаща двойно фосфорилирани Thr 202 и Tyr 204 ERK 1 от човешки произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

За конкурентни изследвания е наличен блокиращ пептид - sc-16982 P, (100 µg пептид в 0.5 ml PBS съдържащ < 0.1% натриев азид и 0.2% BSA).

Приложения: p-ERK 1/2 (Thr 202/Tyr 204) се препоръчва за доказване на Thr 202 и Tyr 204 фосфорилиран p-ERK 1 и Thr 185 и Tyr 187 фосфорилиран ERK 2 от миши, плъши и човешки произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500), Имунохистохимия (включително парафинови срези) (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Молекулна маса на p-ERK 1: 44 kDa.

Молекулна маса на p-ERK 2: 42 kDa.

Положителна контрола: HeLa + UV клетъчен лизат: sc-2221, HeLa + TNFα клетъчен лизат: sc-2228 или NIH/3T3 клетъчен лизат: sc-2210..

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

MEL-1B-R (H-18): sc-13174

Обща информация: ----------

Хромозомна локализация: Генетичен локус: MTNR1B (човек) - 11q14.3.

Източник: MEL-1B-R (H-18) е афинитетно пречистено козе поликлонално антитяло получено срещу пептид във вътрешния регион на MEL-1B-R от човешки произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

За конкурентни изследвания е наличен блокиращ пептид - sc-13174 P, (100 µg пептид в 0.5 ml PBS съдържащ < 0.1% натриев азид и 0.2% BSA).

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Приложения: MEL-1B-R (H-18) се препоръчва за доказване на MEL-1B-R от човешки произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Подходящо за използване като контролно антитяло за MEL-1B-R siRNA (h): sc-40114, MEL-1B-R shRNA Plasmid (h): sc-40114-SH и MEL-1B-R shRNA (h) Lentiviral Particles: sc-40114-V..

Молекулна маса на MEL-1B-R: 36 kDa.

Положителна контрола: COLO 205 тотален клетъчен лизат: sc-364177.

Препоръчани вторични реактиви: За постигане на оптимални резултати се препоръчват следните вторични реактиви:
1) Имуноблот: използвайте матарешко анти-козе IgG-HRP: sc-2020 (диапазон на разреждане: 1:2000-1:100,000) или Cruz Marker™ съвместимо магарешко анти-козе IgG-HRP: sc-2033 (диапазон на разреждане: 1:2000-1:5000), Cruz Marker™ стандарт за молекулна маса: sc-2035, TBS Blotto A Блокиращ Реактив: sc-2333 и Имуноблот Луминол Реактив: sc-2048.
2) Имунопреципитация: използвайте Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).
3) Имунофлуоресценция: използвайте магарешко анти-козе IgG-FITC: sc-2024 (диапазон на разреждане: 1:100-1:400) или магарешко анти-козе IgG-TR: sc-2783 (диапазон на разреждане: 1:100-1:400) с UltraCruz™ среда за покриване: sc-24941.

Поз.7.21.
Превод от английски език

Antibodies-Online

Caspase 3, Apoptosis-Related Cysteine Peptidase (CASP3) antibody

Антиген:	Caspase 3, Apoptosis-Related Cysteine Peptidase (CASP3)
Синоними:	xcpp32, Lice, CPP32, SCA-1, CPP32B, CC3, CG14902, Cas3, Casp 3, Casp3, Caspase-3, DECAY, Decay, Dmel\\CG14902, Drosophila executioner caspase related to Apopain/Yama, caspase 3, caspase-3, CG7788, DRICE, Dmel\\CG7788, DrICE, DrIce, Drice, ICE, crice, drICE, drIce, drice, ice
Епитоп:	Карбоксилен край
Реактивност:	Човек
Гостоприемник:	Заек
Клоналност:	Поликлонално
Приложение:	Имуноблот (WB), ELISA, Имунохистохимия
Каталожен номер:	ABIN346985
Количество:	200 µl
Наличност:	.....

Информация за продукта

Имуноген:	Синтетичен пептид получен от карбоксилния край на човешка цистеинова протеаза CPP32
Изотип:	IgG
Специфичност:	Реагира с човешка 32 kDa Caspase-3 в клетъчни екстракти
Кръстосана реактивност:	Реагира с човешка 32 kDa Caspase-3 в клетъчни екстракти
Пречистване:	Антисерум
Общи данни:	----------

Детайли на приложение

Бележки за прилагане:	Оптималното разреждане трябва да се определи от потребителя. Препоръчвани разреждания: Имунохистохимия – 1/100-1/500, Имуноблот 1:500-5000
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Лиофилизиран
Разтваряне:	Трябва да се разтвори с дестилирана вода
Консерванти:	натриев азид
Съхранение:	4 °C /-20 °C

Поз.7.22.
Превод от английски език

BioVision

LOX-1 поликлонално антитяло

Каталожен №:	3659-100
Количество:	100 µl
Формулировка:	100 µl афинитетно пречистено козе анти-LOX-1 рполиклонално антитяло във фосфатно буфериран разтвор (PBS), pH 7.2, съдържащ 50% глицерол, 1% BSA, 0.02% тримерозал
Видова реактивност:	Човек (други не са тествани)
Съхранение:	Съхранявайте при -20oC. За по-дълъг период, разфасовайте и замразете на -70oC. Избягвайте повторно замразяване и размразяване
Общи данни:	.....
Приложение и употреба:	Антитялото може да се използва за Имуноблот (1:500-1000) използвайки ECL. Обаче, оптималните условия трябва да се определят индивидуално. Други приложения не са определяни.
Забележка	Само за изследователски цели! Да не се използва на хора!

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

TLR4 (H-80): sc-10741

Обща информация:	---
Хромозомна локализация:	Генетичен локус: TLR4 (човек) – 9q33.1; Tlr4 (мишка) – 4 C1
Източник:	TLR4 (H-80) е заешко поликлонално антитяло, получено срещу аминокиселини 242-321 на TLR4 от човешки произход.
Продукт:	Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.
Приложения:	TLR4 (H-80) се препоръчва за доказване на TLR4 от човешки и в по-малка степен от миши и плъши произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000). Подходящо за използване като контролно антитяло за TLR4 siRNA (h): sc-40260, TLR4 siRNA (m): sc-40261, TLR4 siRNA (r): sc-156001, TLR4 shRNA Plasmid (h): sc-40260-SH, TLR4 shRNA Plasmid (m): sc-40261-SH, TLR4 shRNA Plasmid (r): sc-156001-SH, TLR4 shRNA (h) Lentiviral Particles: sc-40260-V, TLR4 shRNA (m) Lentiviral Particles: sc-40261-V и TLR4 shRNA (r) Lentiviral Particles: sc-156001-V. Молекулна маса на TLR4: 95 kDa. Молекулна маса на глшкозилиран TLR4: 120 kDa. Положителна контрола: HEL 92.1.7 клетъчен лизат: sc-2270, THP-1 клетъчен лизат: sc-2238 или HL-60 тотален клетъчен лизат: sc-2209
Съхранение:	Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.
Изследователска употреба:	Само за изследователски цели, а не за диагностични процедури

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

Винкулин (N-19): sc-7649

Обща информация: ----------

Хромозомна локализация: Генетичен локус: VCL (човек) – 10q22.2; Vcl (мишка) – 14 A3

Източник: Винкулин (N-19) е афинитетно пречистено козе поликлонално антитяло, получено срещу пептиден участък в N-терминалния край на винкулин от човешки произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

За провеждане на конкурентни изследвание е наличен блокиращ пептид, sc-7649-P (100 µg пептид в 0.5 мл. PBS, съдържащ <0.1% натриев азид и 0.2% BSA)

Приложения: Винкулин (N-19) се препоръчва за доказване на винкулин от миши, плъши, човешки и *Drosophila melanogaster* произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Винкулин (N-19) също се препоръва за доказване на винкулин в допълнителни видове, включващи кон, куче, говедо, свиня и птица.

Подходящо за използване като контролно антитяло за винкулин siRNA (h): sc-29524, винкулин siRNA (m): sc-36819, винкулин shRNA Plasmid (h): sc-29524-SH, винкулин shRNA Plasmid (m): sc-36819-SH, винкулин shRNA (h) Lentiviral Particles: sc-29524-V и винкулин shRNA (m) Lentiviral Particles: sc-36819-V.

Молекулна маса на Винкулин: 117 kDa.

Положителна контрола: HeLa тотален клетъчен лизат: sc-2200, HISM клетъчен лизат: sc-2229 или винкулин (h): 293T лизат: sc-113822.

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури

Поз.7.25.
Превод от английски език

Antibodies-Online

Integrin beta 1 (ITGBl) antibody

Антиген:	Integrin beta 1 (ITGBl)
Синоними:	CD29, FNRB, MDF2, VLAB, GPIIA, MSK12, VLA-BETA, 4633401G24Rik, AA409975, AA960159, ENSMUSG00000051907, Fnrb, Gm9863, gplla, cd29, fnrb, gpiia, itgbl, mdf2, msk12, via-beta, vlab
Реактивност:	Мишка
Гостоприемник:	Плъх
Клоналност:	Моноклонално (9EG7)
Приложение:	Флоуцитометрия, Имунохистохимия на парафинови срези, Имуноблот, Имунопреципитация, Функционални изследвания
Каталожен номер:	ABIN967378
Количество:	0.5 mg
Концентрация	0.5 mg/ml
Наличност:	.....

Информация за продукта

Имуноген:	Миша ендотелна клетъчна линия
Клон:	9EG7
Изотип:	IgG2a капа
Пречистване:	Пречистено от супернатанта на тъканни култури или асцит, посредством афинитетна хроматография
Чистота	Пречистено
Общи данни:	----------

Детайли на приложение

Бележки за прилагане:	Този продукт се използва за: Имуноблот, Имунохистохимия на парафинови срези, Имуноцитохимия. При Имуноблот, антитялото открива ивица с приблизителна маса от 17kDa. Препоръчвани разреждания: Имунохистохимия / Имунофлуоресценция 1:100-250, Имунохистохимия 1:10-1:500, Имунохистохимия на парафинови срези 1:250, Имуноблот 1:1000-10000
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Течен
Буфер:	PBS 49 % , Glycerol 50 % , BSA 0.05 % , Sodium Azide
Консерванти:	натриев азид
Съхранение:	4 °C

PLOS | ONE

The Primacy of β1 Integrin Activation in the Metastatic Cascade

Hisashi Kato, Zhongji Liao, John V. Mitsios, Huan-You Wang, Elena I. Deryugina, Judith A. Varner, James P. Quigley, Sanford J. Shattil

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Abstract

After neoplastic cells leave the primary tumor and circulate, they may extravasate from the vasculature and colonize tissues to form metastases. β1 integrins play diverse roles in tumorigenesis and tumor progression, including extravasation. In blood cells, activation of β1 integrins can be regulated by "inside-out" signals leading to extravasation from the circulation into tissues. However, a role for inside-out β1 activation in tumor cell metastasis is uncertain. Here we show that β1 integrin activation promotes tumor metastasis and that activated β1 integrin may serve as a biomarker of metastatic human melanoma. To determine whether β1 integrin activation can influence tumor cell metastasis, the β1 integrin subunit in melanoma and breast cancer cell lines was stably knocked down with shRNA and replaced with wild-type or constitutively-active β1. When tumor cells expressing constitutively-active β1 integrins were injected intravenously into chick embryos or mice, they demonstrated increased colonization of the liver when compared to cells expressing wild-type β1 integrins. Rescue expression with mutant β1 integrins revealed that tumor cell extravasation and hepatic colonization required extracellular ligand binding to β1 as well as β1 interaction with talin, an intracellular mediator of integrin activation by the Rap1 GTPase. Furthermore, shRNA-mediated knock down of talin reduced hepatic colonization by tumor cells expressing wild-type β1, but not constitutively-active β1. Overexpression in tumor cells of the tumor suppressor, Rap1GAP, inhibited Rap1 and β1 integrin activation as well as hepatic colonization. Using an antibody that detects activated β1 integrin, we found higher levels of activated β1 integrins in human metastatic melanomas compared to primary melanomas, suggesting that activated β1 integrin may serve as a biomarker of invasive tumor cells. Altogether, these studies establish that inside-out activation of β1 integrins promotes tumor cell extravasation and colonization, suggesting diagnostic and therapeutic approaches for targeting of β1 integrin signaling in neoplasia.

Figures

Citation: Kato H, Liao Z, Mitsios JV, Wang H-Y, Deryugina EI, et al. (2012) The Primacy of β1 Integrin Activation in the Metastatic Cascade. PLoS ONE 7(10): e46576. doi:10.1371/journal.pone.0046576

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Introduction

The process of cancer metastasis involves a cascade of events beginning at the primary tumor where neoplastic cells breakdown the extracellular matrix, migrate and intravasate into the vasculature [1]–[3]. Circulating tumor cells may be escorted and modified by platelets [4] and myeloid cells [5], and the metastatic process proceeds by tumor cell extravasation through blood vessels, and by seeding and colonization of a compatible niche within a distant organ [6] or even within the primary tumor [7], [8]. Tumor cells must negotiate a veritable gauntlet of environmental influences for procession through these steps of the metastatic cascade.

One mechanism that tumor cells employ during tumor progression is regulation of adhesion receptor expression [9], [10]. For example, reciprocal expression of cadherins and integrins promotes epithelial-to-mesenchymal transition [11]. Integrin αβ heterodimers expressed by tumor cells interact with extracellular matrix ligands or cellular counter-receptors to influence cell adhesion, migration, proliferation and survival [10], [12]. Within this context, the β1 integrin subunit is almost universally expressed in tumor cells, where interactions with specific matrix ligands, such as collagen, laminin and fibronectin are dictated, in part, by the identity of the integrin α subunit partner [13]. In some human solid tumors, increased expression of certain β1 integrins, for example α2β1 [14], α3β1 [15], [16], α5β1 [17], [18], or α6β1 [19], correlates with increased metastatic potential [20]–[23], and in some cases with shortened patient survival [17], [24]–[30]. On the other hand, α2β1 may suppress the progression of certain tumors [31], [32]. The therapeutic potential of β1 integrin blockade in cancer has led to current investigations of selective β1 inhibitors in animal models [33] and early clinical trials [34].

One aspect of β1 integrin function that has received relatively little attention in the cancer field is "inside-out" activation, whereby intracellular signals rapidly regulate integrin affinity for ligands through conformational changes propagated from the integrin cytoplasmic tails and transmembrane domains to the extracellular domains [35]. Thus, whereas changes in cell surface β1 integrin expression may take many minutes when regulated by receptor cycling and hours when regulated by transcription, integrin activation can take place within seconds, theoretically placing tumor cells at a relative advantage in metastatic tumor formation. Inside-out integrin signaling has been studied primarily in blood cells where β2 [36] and β3 integrin activation [37]–[39] are required for normal leukocyte trafficking and platelet aggregation, respectively. While β1 integrins are also subject to inside-out regulation in platelets [39]–[41], the role of β1 integrin activation in non-hematopoietic cells, and solid tumor cells in particular, remains to be clarified.

Based on these considerations, the current studies were carried out to investigate whether activation of β1 integrins in human tumor cells can modulate the metastatic process. We focused on the later stages of the metastatic cascade, analyzed primary and metastatic human tumors, and employed two complementary vertebrate experimental metastasis model systems. Our results establish that activated β1 integrins are expressed in certain human tumors, and that inside-out signaling to β1 integrins can determine the success or failure of tumor cell extravasation and metastatic colonization.

Results

Activated β1 Integrins Promote Hepatic Colonization by Tumor Cells in Experimental Metastasis Assays

To begin to address a potential role for β1 integrin activation in tumor metastasis, genetically-engineered MDA-MB435 human melanoma cells were injected into the venous circulation of chick embryos and colonization to the liver was evaluated five days later. This model was employed because it is relatively rapid, enables facile

quantification of human tumor cell colonization using human *Alu*-specific real-time PCR, and our preliminary experiments established that hepatic colonization in this system is dependent on β1 integrins (Fig. S1), but not β3 integrins (Fig. S2). When β1 integrin activation in MDA-MB435 cells was quantified by flow cytometry using antibody 9EG7, basal antibody binding to cells expressing constitutively-active β1-L358A was greater than the binding to cells expressing wild-type β1 ($P<0.01$) (Fig. 1A). Consistent with this, the adhesion of β1-L358A MDA-MB435 cells to low plating concentrations of collagen or laminin was increased (Fig. 1B, C), despite the fact that β1-L358A expression was approximately 50% that of wild-type β1 (Fig. 1A). There was no difference in the growth of primary tumors on the chick chorioallantoic membrane when cells expressing wild-type β1 and β1-L358A were compared (Fig. 1D). However, when MDA-MB435 tumor cells were injected intravenously into the chick embryo, the number of β1-L358A cells detected in the liver five days later was increased compared to cells expressing wild-type β1 (Fig. 1E) ($P<0.01$). This result was not confined to melanoma cells because it was also observed with genetically-engineered MDA-MB231 breast cancer cells expressing constitutively-active β1 integrin (Fig. 1F). Furthermore, the results were not confined to the chick model system because increased macroscopic hepatic metastases were observed when B16F10 mouse melanoma cells expressing β1-L358A were injected into the mouse splenic vasculature and livers examined seven days later (Fig. 1G). Thus, expression of activated β1 integrins endows circulating tumor cells with a selective advantage in hepatic colonization.

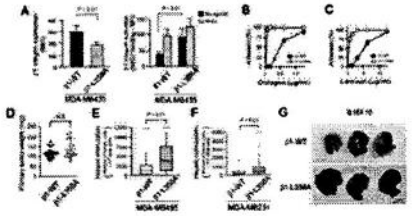


Figure 1. Activated β1 integrins promote hepatic colonization by tumor cells in experimental metastasis assays.
(A) β1 integrin expression and activation determined by flow cytometry. Expression of β1 integrins in MDA-MB435 melanoma cells was determined by binding of antibody TS2/16. Activation of β1 integrins was determined by binding of antibody 9EG7 (basal: black bar, $MnCl_2$ stimulation: gray bar). Data represent mean fluorescence intensity in arbitrary fluorescence units (MFI) ± SEM (n = 8). (B and C) Tumor cell adhesion to collagen (B) and laminin (C). β1 wild-type (WT) and β1-L358A cells were incubated in 96 well plates coated with the indicated input concentrations of collagen or laminin for 60 minutes at 37°C. Data are expressed as cell adhesion normalized to total input of cells. Data represent means ± SEM (n = 3). (D) Weight of primary tumors in the chick chorioallantoic membrane. 10^6 β1-WT (n = 25) or β1-L358A MDA-MB435 cells (n = 20) were resuspended in Matrigel and inoculated on top of the membrane of 10 day-old eggs. Tumor weight was measured after seven days of incubation. (E and F) Hepatic colonization in the chick embryo experimental metastasis assay. Box plots for tumor cell colonization in the chick embryo liver five days after intravenous injection. (E) MDA-MB435 melanoma cells (β1-WT: n = 50 livers, β1-L358A: n = 32 livers) and (F) MDA-MB231 breast cancer cells (β1-WT: n = 46 livers, β1-L358A: n = 51 livers). Human tumor cells in the chick embryo liver were quantified as described in Materials and Methods. (G) Representative images of hepatic melanoma metastases in a mouse experimental metastasis system seven days after intrasplenic injection of the indicated B16F10 mouse melanoma cells. Black, melanin-containing metastatic lesions were evident macroscopically.
doi:10.1371/journal.pone.0046576.g001

β1 Integrins must be Competent to Bind Extracellular Ligands to Promote Hepatic Colonization by Tumor Cells

Since activated β1 integrins bind ligands with enhanced affinity, they might well be expected to affect tumor cell adhesion and motility during steps of the metastatic cascade. However, some aspects of tumor progression may be influenced by integrins in a ligand independent manner [42]. To address whether ligand binding to β1 integrins is necessary for hepatic colonization, MDA-MB435 melanoma cells expressing wild-type β1 were compared in the chick experimental metastasis system to cells expressing equivalent levels of β1-D130A (Fig. S3A), a point mutant with impaired ligand binding [43]. As expected, the adhesion of β1-D130A melanoma cells to collagen or laminin was markedly impaired (Fig. S3B), whether or not adhesion was studied in the presence of 0.5 mM $MnCl_2$ to extrinsically activate integrins (Fig. S3C). Despite this, growth of primary tumors on the chick embryo chorioallantoic membrane was not affected by the β1-D130A mutation (Fig. 2A). However, when β1-D130A cells were injected into the chick embryo venous circulation, hepatic colonization was markedly reduced compared to cells expressing wild-type β1 (Fig. 2B). Thus, activation of and ligand binding to β1 integrins are required for hepatic colonization by circulating MDA-MB435 melanoma cells but not for the growth of these cells when implanted on the chorioallantoic membrane. When specific α integrin subunits known to partner with β1 were knocked down in MDA-MB435 cells (Fig. S4A), those deficient in the α2 subunit exhibited the most profound decrease in hepatic colonization after intravenous injection into the chick embryo (Fig. S4B), suggesting that the collagen receptor, α2β1, is a major β1 integrin to promote later stages of metastasis in this system.

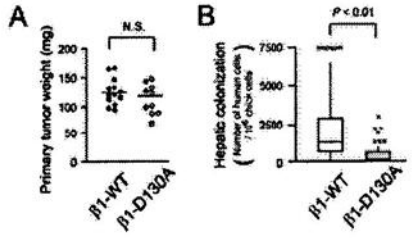


Figure 2. Ligand binding to β1 integrins is required for hepatic colonization by tumor cells.
(A) In vivo tumor growth assay on the chick chorioallantoic membrane. Tumors growing on the membrane were weighed seven days after inoculation of 10^6 β1-WT (n = 13) or β1-D130A MDA-MB435 cells (n = 10). (B) Box plot showing the number of β1-WT (n = 67) and β1-D130A cells (n = 57) quantified in chick embryo livers five days after intravenous tumor cell injection.
doi:10.1371/journal.pone.0046576.g002

Inside-out Regulation of β1 Integrin Activation Affects Hepatic Colonization by Tumor Cells

Integrin activation requires interaction of the β cytoplasmic tail with talin. Therefore, to investigate a role for inside-out activation of β1 integrins in the process of hepatic colonization, a mutation (I782A) was introduced into the β1 cytoplasmic tail that disrupts talin interaction with β1 [44]. Disruption of talin binding by this mutation was confirmed in a pull-down assay using recombinant β1 tail peptides (Fig. S5A). Furthermore, MDA-MB435 cells expressing β1-I782A showed impaired adhesion to collagen and laminin in the absence of $MnCl_2$ (Fig. S5B), but not in the presence of $MnCl_2$ to extrinsically activate the integrin (Fig. S5C). Cells expressing β1-I782A were able to grow normally when implanted on the chick chorioallantoic membrane (Fig. 3A), but they could not promote hepatic colonization when injected intravenously (Fig. 3B). Simultaneous incorporation of the L358A activating mutation into β1-I782A restored hepatic colonization by MDA-MB435 cells (Fig. 3C), consistent with the notion that talin-dependent inside-out signaling to β1 was required for colonization and had been impaired by the I782A mutation. To specifically study the role of talin binding to β1 in tumor cell extravasation, hepatic sinusoids near the surface of the chick embryo liver were evaluated by two-photon microscopy 24 hours after intravenous injection of fluorescently-labeled tumor cells. While over 55% of cells expressing wild-type β1 had extravasated from sinusoids by this time, only ~25% of the β1-I782A cells had done so ($P<0.01$) (Fig. 3D). Furthermore compared to tumor cells expressing wild-type β1, those expressing β1-I782A exhibited decreased total numbers in the liver 24 hours after intravenous injection (Fig. S6). Collectively, these results suggest that talin binding to β1 integrins is required for tumor cell extravasation and colonization.

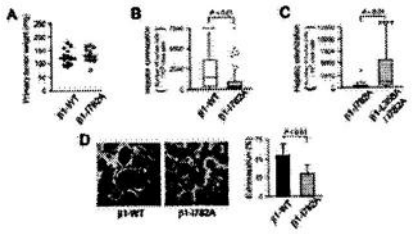


Figure 3. Inside-out activation of β1 integrin is required for hepatic colonization by tumor cells.
(A) In vivo tumor growth assay on the chick chorioallantoic membrane. Tumors growing on the membrane were weighed seven days after inoculation of 10^6 β1-WT (n = 18) or β1-I782A MDA-MB435 cells (n = 13). (B and C) Box plots showing the number of tumor cells colonized to the chick liver five days after intravenous injection. (B) β1-WT (n = 34) and β1-I782A cells (n = 28) and (C) β1-I782A (n = 28) and β1-L358A/I782A cells (n = 32). (D) Tumor cell extravasation from liver sinusoids. Twenty-four hours after intravenous injection of tumor cells labeled with tdTomato (red), chick embryo livers were imaged using a two-photon microscope after labeling hepatic vasculature with FITC-lectin (green). Representative images of livers from embryos injected with β1-WT or β1-I782A tumor cells depict an extravasated cell in the left panel and intravascular cell in the right panel. Three-dimensional images were digitally reconstructed and the percentage (± SEM) of extravasated cells was quantified (β1-WT: 222 cells, β1-I782A: 142 cells; six independent experiments).
doi:10.1371/journal.pone.0046576.g003

To study talin directly, the protein was knocked down in MDA-MB435 with shRNA. Each of three shRNA constructs decreased talin expression (Fig. S7A), without substantially affecting β1 integrin expression (Fig. S7B). Talin knockdown decreased the adhesion of cells expressing wild-type β1 to collagen and laminin, but not the adhesion of cells expressing constitutively-active β1-L358A (Fig. S7C, D). Moreover, talin knock down decreased hepatic colonization by tumor cells expressing wild-type β1 ($P<0.01$) (Fig. 4A), but not colonization by cells expressing β1-L358A (Fig. 4B). Talin contains an N-terminal head domain and a C-terminal rod domain, and recombinant expression of the integrin-binding head domain can directly activate integrins from inside cells [45]. Indeed, overexpression of the talin head domain increased hepatic colonization by talin knock down cells expressing wild-type β1, whereas a talin head domain mutant (W359A) incapable of binding to β1 [46] failed to do so (Fig. 4C). Since the effect of the talin head domain required both the expression (Fig. 4D) and ligand binding capacity of β1 integrins (Fig. 4E), these results imply that inside-out regulation of talin binding to and activation of β1 integrins promotes hepatic colonization by tumor cells.

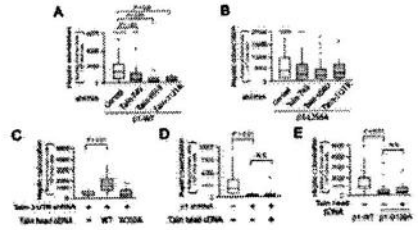
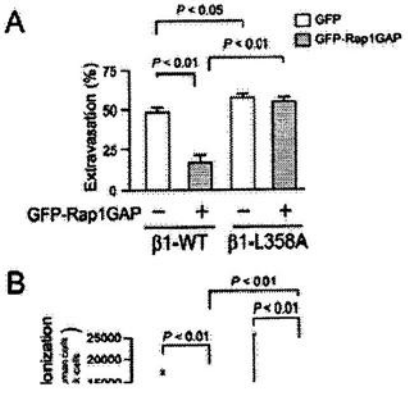


Figure 4. Talin-mediated β1 integrin activation is required for hepatic colonization by tumor cells.
Hepatic colonization in the chick embryo experimental metastasis model. (A and B) Box plots showing the effect of talin knockdown in MDA-MB435 cells. Lentivirus encoding a control shRNA or one of three talin shRNAs were transduced into (A) MDA-MB435 β1-WT cells or (B) β1-L358A cells. Box plots depict numbers of human tumor cells quantified in the liver five days after intravenous injection into chick embryos. (C) Effect of wild-type or W359A talin head domain on hepatic colonization in talin knock down cells. (D) Talin head domain cannot rescue the blocking effect of β1 integrin knock down on hepatic colonization (control: n = 14, β1shRNA: n = 15, β1shRNA + talin head: n = 19). (E) Talin knock down cannot rescue the blocking effect of ligand binding-defective β1-D130A on hepatic colonization (β1-WT: n = 27, β1-D130A: n = 23, β1-D130A + talin head: n = 19).
doi:10.1371/journal.pone.0046576.g004

A Tumor Suppressor Gene can Regulate β1 Integrin Activation and Hepatic Colonization by Tumor Cells

While mutational activation of integrins in human cancer is not commonly reported, β1 integrin activation in tumor cells might be promoted by 1) stimulation of inside-out signaling through oncogenic growth factor receptor pathways, and/or 2) deletion of a tumor suppressor gene that normally functions to dampen integrin activation. One such potential tumor suppressor is Rap1GAP, which converts active Rap1-GTP to inactive Rap1-GDP, and is deleted in a number of cancers, including melanoma [47]. Since Rap1 mediates talin-dependent integrin activation [35], [48], we studied the effect of GFP-Rap1GAP on the formation of hepatic colonization. Expression of GFP-Rap1GAP in MDA-MB435 cells decreased both the levels of Rap1-GTP and cell adhesion dependent on β1 integrin (Fig. S8A, B). Moreover following intravenous injection, tumor cells expressing both wild-type β1 integrin and GFP-Rap1GAP exhibited less extravasation into the liver parenchyma (Fig. 5A) and less hepatic colonization (Fig. 5B) compared to cells expressing only wild-type β1 ($P<0.01$). However, while constitutively-active β1 integrin L358A rescued the suppressive effect of over-expressed Rap1GAP on tumor cell extravasation (Figure 5A), it was less able to rescue the suppressive effect of Rap1GAP on hepatic colonization (Figure 5B). Thus, Rap1GAP may also exert a β1 integrin activation-independent effect on tumor cell colonization after extravasation has occurred (for example on tumor cell survival, proliferation or apoptosis). Overall, these results indicate that the later stages of the metastatic cascade can be modulated by a tumor suppressor within a Rap1 signaling pathway that controls β1 integrin activation.



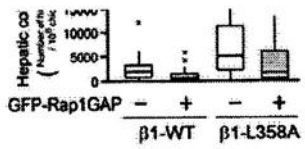


Figure 5. Rap1GAP suppresses hepatic colonization of tumor cells.
(A) Tumor cell extravasation from liver sinusoids. Tumor cell extravasation was determined as in Figure 3 (β1-WT + GFP: 168 cells, β1-WT + GFP-Rap1GAP: 101 cells, β1-L358A + GFP: 84 cells, β1-L358A + GFP-Rap1GAP: 144 cells; five independent experiments). (B) Box plot showing the effect of Rap1GAP overexpression on hepatic colonization by MDA-MB435 β1-WT or β1-L358A cells (β1-WT + GFP: n = 28, β1-WT + GFP-Rap1GAP: n = 31, β1-L358A + GFP: n = 30, β1-L358A + GFP-Rap1GAP: n = 29).
doi:10.1371/journal.pone.0046576.g005

Human Metastatic Tumors Express Activated β1 Integrins

Our findings from animal models indicate a role for inside-out activation of β1 integrins in the later stages of the metastatic cascade. To begin to address a potential role for β1 integrin activation in human tumor metastasis, we assessed β1 integrin activation in formalin-fixed, paraffin-embedded (FFPE) sections of two common solid tumors, breast cancer and melanoma by using monoclonal antibody 9EG7 as a reporter for ligand-bound and activated β1 [49], [50]. The validity of using 9EG7 for this purpose was assessed in preliminary studies with genetically-engineered MDA-MB435 melanoma cells stained with both 9EG7 and antibody 4B7R (for total β1). While 9EG7 stained a relatively small sub-population of 4B7R-positive cells expressing wild-type β1, it stained the majority of cells expressing constitutively-active β1-L358A (Fig. S9). When human tumor samples were stained with 9EG7, we found a sub-population of β1 to be activated in primary and metastatic breast cancer and melanoma (Fig. 6A, B). When the tumor-bearing areas stained with antibodies 9EG7 and 4B7R were compared in a relatively large number of available melanoma samples, the proportion of activated β1 was increased in metastatic tumors compared to primary tumors ($P<0.01$) (Fig. 6C). Similarly, the fluorescence signal intensity of activated β1 relative to total β1 was increased in the metastatic tumors (median activated β1 signal intensity/total β1 intensity: 0.55 in primary tumors and 0.71 in metastatic tumors, $P<0.01$). These results suggest a role for β1 integrin activation in the metastatic cascade.

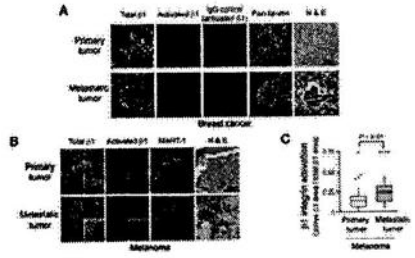


Figure 6. Activated β1 integrins in primary and metastatic human tumors.
(A and B) Representative immunostaining of FFPE tissue sections for total β1 integrin (antibody 4B7R) and activated β1 integrin (antibody 9EG7) in primary and metastatic tumors. For detection and analysis of tumor cells, samples were stained with an antibody to pan-keratin (breast cancer) (A) or MART-1 (melanoma) (B). The breast cancer metastasis was from a lymph node and the melanoma metastasis was from the abdominal cavity. Primary and metastatic tumors were from different patients. Note that in the primary breast tumor and metastatic melanoma, activated β1 integrins were observed in some connective tissue cells as well as in tumor cells. Fluorescence images of primary and metastatic tumors were processed identically. (C) Box plot showing β1 integrin activation in primary ($n = 50$) and metastatic ($n = 26$) melanomas in a tissue microarray. The proportion of areas with activated β1 integrin to total β1 integrin within MART-1 positive melanoma cells was analyzed with Volocity software. Statistical analysis was performed by Mann-Whitney U test (Outliers, values outside of 1.5 X interquartile distance from the quartiles, are indicated by an X).
doi:10.1371/journal.pone.0046576.g006

Discussion

In the present study, we have investigated the role of inside-out signaling and activation of β1 integrins in later stages of the metastatic cascade. Given previous studies indicating the involvement of β1 integrins in multiple aspects of tumorigenesis and progression, both in animal models and in human cancers, we posited that the *activation state* of tumor cell β1 integrins, and not just the their level of expression, could impact later events in the metastatic cascade. By studying primary and metastatic human tumors and tumor cell extravasation and metastatic colonization in two vertebrate models of experimental metastasis, the following conclusions can be drawn: 1) A sub-population of β1 integrins in primary and metastatic human breast cancer and melanoma is expressed in an activated state, and in the case of melanoma, where sufficient numbers of patient samples were available for analysis, metastatic tumors expressed relatively more activated β1 integrins than primary tumors. 2) Activated β1 integrins can promote human tumor cell extravasation from the vasculature and metastatic colonization of the liver in animal models. 3) The activation state of β1 integrins in tumor cells, and subsequent extravasation and colonization, are regulated by a canonical inside-out integrin signaling pathway that includes Rap1 and talin and that requires interaction of talin with the β1 integrin cytoplasmic tail. 4) β1 integrin activation in tumor cells can be regulated by a tumor suppressor, Rap1GAP, implying that one mechanism by which this protein may affect later stages of the metastatic cascade is modulation of Rap1-dependent inside-out integrin signaling.

Increased β1 integrin expression is a prognostic factor in some human tumors [17], [24]–[30]. The present study showing relatively greater activation of β1 in human melanoma metastases compared to primary tumors suggests that future studies should investigate the activation state of β1 integrins as a potential prognostic marker in human cancers. The β1 activation-dependent antibody used in the present work detects high-affinity β1 integrins, but it also reports on ligand occupancy of the integrins and likely on integrin clustering (avidity) [50]. It is clear from our experimental metastasis studies that inside-out signaling and affinity modulation of β1 integrins can be determinants of tumor cell extravasation and colonization. However, since mechanisms of affinity and avidity regulation may differ in fine detail, future studies should also address the possible role of integrin clustering in metastasis when quantitative methods become available to differentiate β1 affinity and avidity modulation in tumor samples.

In previous mouse experiments with human breast cancer cells, the activation state of integrin αVβ3 correlated with distant metastasis [51]. In our chick embryo model system, hepatic colonization was dependent on β1 integrins, but not on αVβ3. Since tumor cell αVβ3 promotes not only tumor cell arrest in vessels but also tumor cell interaction with platelets [51], [52], the precise phases of the metastatic cascade influenced by β1 and β3 integrins may differ. For example, platelets via β3 integrins may escort and help to phenotypically reprogram circulating tumor cells [4], [53], effects not likely to be mediated by the relatively small number of β1 integrins expressed in platelets. The present studies, by focusing primarily on experimental hepatic colonization, do not address whether β1 integrin activation might also affect later stages of the metastatic cascade involving other organs or earlier stages of the cascade before cells enter the circulation from the primary tumor. However, our analysis of a human melanoma tumor array (Fig. 6C) suggests the possibility that metastases to lymph nodes and other sites may be affected by inside-out signaling to β1 integrins. While the experimental results demonstrate specific effects of β1 integrin activation on tumor cell extravasation and colonization (Fig. 3D), a limitation of the work in extrapolating to metastases in humans is that our direct intravascular injection of tumor cells in animal models does not reflect the process of cell

intravasation from primary tumors. In addition, certain later processes in the metastatic cascade, such as migration within the extracellular matrix of the metastatic niche and tumor cell dormancy might be influenced by β1 integrin activation, but they were not investigated here.

The activation state of β1 integrins in tumor cells, and subsequent extravasation and metastatic colonization, were found to be regulated by an inside-out integrin signaling pathway that includes Rap1 and talin and requires interaction of talin with the β1 integrin cytoplasmic tail. Thus, the integrin activation paradigm, worked out largely with studies in hematopoietic cells [38]–[40], [54], [55], may also be relevant to circulating solid tumor cells, perhaps after they have undergone epithelial-to-mesenchymal transition. Thus, a role for β1 integrin activation may explain, in part, recent experimental work demonstrating the effects on tumor progression of manipulating the expression of Rap1, the Rap1 effector, RIAM [56], Rap1GAP [57] and talin [58]. In this regard, it is interesting to note that some members of the kindlin protein family [59]–[61], which bind integrin β tails and regulate talin-dependent integrin activation [62], [63], have also been implicated in solid tumor metastasis.

We found that β1 integrin activation in tumor cells and hepatic colonization was reduced by overexpression of the Rap1GAP tumor suppressor (Fig. 5). This suggests that one function of some tumor suppressors may be to hold integrin activation in check, an idea supported by the recent identification of a number of other putative tumor suppressors that may act at the level of integrin activation [64]–[66]. Consequently, it may be productive to move beyond β1 integrin blockade or manipulation of β1 expression as a cancer therapeutic strategy and consider the inside-out integrin activation process in tumor cells as a feature-rich set of potential therapeutic targets to limit the metastatic cascade.

Materials and Methods

Ethics Statement

Analysis of human breast cancer samples was approved by the UCSD Human Research Protections Program (IRB# 080911). Samples were collected as part of diagnostic or therapeutic surgery after patients gave written informed consent. No patient identifying data were available during this study. Animal experiments were conducted under a protocol approved by the University of California, San Diego Animal Subjects Committee.

Antibodies, Cell Lines and Culture

Monoclonal antibodies to human β1 (clone TS2/16), α1, α2, α3, α5, and α6 integrin were from BioLegend (San Diego, CA). Another monoclonal antibody to human β1 integrin (clone 4B7R) was from AbD Serotec (Raleigh, NC). Monoclonal antibody to activated β1 integrin (clone 9EG7) [49] was from BD Biosciences (San Jose, CA). Polyclonal antibody to cytokeratin was from Dako (Carpinteria, CA). Monoclonal antibody to talin (clone 8D4) [45], [67] and β-actin were from Sigma Aldrich (St Louis MO). Polyclonal antibody for Rap1 was from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibody for MART-1 and polyclonal antibody for human CD44 were from Spring Bioscience (Pleasonton, CA). Polyclonal antibody to cleaved caspase-3 was from Trevigen (Gaithersburg, MD). Human cancer cell lines (breast: MDA -MB231; melanoma: MDA-M435, provided by Richard Klemke, UCSD) and the mouse melanoma cell line B16F10 (provided by Mark Ginsberg, UCSD, American Tissue Culture Collection CRL-6475) were grown in DMEM supplemented with 10% fetal bovine serum, L-glutamine and antibiotics at 37°C and 5% CO_2.

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Lentiviral Vectors for shRNA Knock Downs and Protein Expression

Lentiviral vector FG12-tdTomato and FG12-Puro were generated by substituting GFP in the original FG12 vector [68] with tdTomato [69] (kindly provided by Roger Tsien, University of California, San Diego) or the puromycin resistance gene, respectively. To express short hairpin RNA (shRNA), DNA fragments containing the human U6 promoter and shRNA amplified by PCR were cloned into FG12-tdTomato or FG12-Puro as described [70]. The following were the target sequences for human β1 integrin shRNA: AAGAGTGCCGTAACAACTGTGGTCAATCC; mouse β1 integrin shRNA: ATAAAGATCCTTTCTCAAGTCTTTT; human β3 integrin shRNA: GGCCAGATGATTCGAAGAATT; human talin-749 shRNA (talin head domain): CAATGAGCAGAAGCACAAGGCTGGCTTCC; human talin-6560 shRNA (talin rod domain): CAAGGCCGTTGCTGCTGGCAATTCCTGTC; and human talin-3'UTR shRNA: CCCAGAGTATTAACGCTCCAA; human α1 integrin shRNA: GCCATATGGAGGGAAAGGAAACAGTATGC; α2 integrin shRNA: GCTATATAGTGTGAATGAGAATGGCAATA; α3 integrin shRNA: TGGGACTTATCTGAGTATAGTTACAAGGA; α5 integrin shRNA: TCGAGACAAACTCTCGCCGATTCACATCG; α6 integrin shRNA: GACAGCTCATATTGATGTTCACTTCTTAA.

To rescue β1 integrin expression in β1 integrin knockdown tumor cells, GFP was removed from the original FG12 vector and human β1 integrin cDNA with silent mutations was cloned into FG12. To generate constitutively-active β1 integrin L358A [71], a cDNA fragment containing the L358A mutation (lower case) was amplified by PCR and cloned into the NsiI/StuI site of β1 integrin. 5'–TTGATCATTGATGCATACAATTCCgcgTCCTCAGAAGTCATTTTGG–3'and 5'–AGCCCAGAGGCCTAATCTTGAAGCTGTCAGAATCC–3'. To produce the β1 integrin D130A [43] ligand binding-deficient mutation, a DNA fragment from the start codon to the BglII site of β1 integrin was amplified by PCR and cloned into the pCR2.1-TOPO vector and subcloned into FG12. 5'– AAAACCGGTACCCGCGGAAAAG–3' and 5'–TTTAGATCTGTTCCAAGACTTTTTACATTCTCCAAATCGTCTTTCATTGAGTAAGACAGggcCATAAGGTAGTAGAGG–3'.To introduce the talin binding-deficient I782A [44] mutation in β1 integrin, a cDNA fragment encoding the I782A mutation was prepared by oligo annealing and cloned into the AgeI/AfeI sites of the expression vector. 5'-AATTTGAAAAGGAGAAAATGAATGCCAAATGGGACACcGGTGAAAATCCTgcaTATAAGAGC-3',5'-GCTCTTATAtgcAGGATTTTCACCGGTGTCCCATTTGGCATTCATTTTCTCCTTTTCA-3'.To express wild-type and mutant (W359A) [46] mouse talin head domain, cDNA 3' to the talin F3 sub-domain was removed, and fragments encoding GFP-talin head domain were cloned into FG12 after removal of FG12 GFP segment. To generate lentiviral vector for GFP-Rap1GAP [72], Rap1GAP cDNA was cloned into pEGFP-C1 expression vector and GFP-Rap1GAP was subcloned into FG12. Lentiviruses were generated as described [68].

Flow Cytometry

Cells were resuspended in modified Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 3.3 mM NaH_2PO_4, 1.2 mM $NaHCO_3$, 3.8 mM HEPES, 5.5 mM glucose, 1 mg/ml bovine serum albumin) supplemented with 1 mM $MgCl_2$. To analyze total β1 integrin expression, cells were first incubated with 10 μg/ml antibody TS2/16, followed by washing and incubation with Alexa fluor 647 goat anti-mouse IgG (Invitrogen, Carlsbad, CA). Activated β1 integrin was assessed by incubating with 10 μg/ml antibody 9EG7, followed by washing and incubation with Alexa 647 goat anti-rat IgG (Invitrogen). Surface expression of β3 integrin was analyzed with monoclonal antibody SSA6 [73]. Fluorescence intensity of single, living (propidium iodide-negative) cells was determined by flow cytometry on a FACSCalibur (BD Biosciences, San Jose, CA).

Cell Adhesion, Western Blotting and Pull-down Assays

Ninety-six well plates were coated overnight at 4°C with 100 μl of collagen type I (Sigma Aldrich, St Louis MO) or laminin-1 (Stemgent, San Diego, CA) at increasing concentrations, and then blocked with 1% BSA in phosphate-buffered saline for 1 hour at room temperature. A cell suspension in serum-free DMEM (100 μl; 10^6 cells/ml) was applied to each well and incubated for 1 hour at 37°C in a cell culture incubator. After three washes with phosphate-buffered saline, 100 μl of substrate solution (6 mg/ml p-nitrophenyl phosphate; Sigma Aldrich, St Louis MO) in 50 mM acetic acid, pH 5.0, 1% Triton X-100 were added. After incubation for 1 hour at 37°C, the reaction was stopped with 50 μl of 1N NaOH and optical density was measured in a microplate reader at 405 nm. Cell adhesion was expressed as a percentage of total cells added to the well.

For western blotting, cells were lysed in RIPA buffer (75 mM NaCl, 1% Nonidet P-40, 1% deoxycholic acid, 0.2% sodium dodecyl sulfate, 2.5 mM $MgCl_2$, 1 mM sodium orthovanadate, and proteinase inhibitor cocktail). Proteins were resolved by SDS-polyacrylamide gel electrophorsesis and transferred to nitrocellulose membranes. After blocking with 5% BSA in Tris-buffered saline, membranes were incubated with primary antibodies and then secondary antibodies conjugated to IRDye 680 or IRDye 800CW (LI-COR Biotechnology). Antibody binding was analyzed with the Odyssey imaging system (LI-COR Biotechnology).

For pull-down assays, His-tag recombinant wild-type or mutant β1 integrin cytoplasmic tail model proteins were cloned into pET15b vector, purified and conjugated to neutravidin resin as described [74]. MDA-MB435 cells were solubilized in buffer containing 1% NP-40, 150 mM NaCl, 50 mM Tris pH7.4, 1 mM sodium vanadate, 0.5 mM sodium fluoride, 1 mM leupeptin, and complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). After clarification, 2.0 mg of cell lysate were

incubated with 100 µl of resin overnight at 4°C and bound proteins were resolved by SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting. GTP-bound active Rap1 in MDA-MB435 cells was detected by pull-down assay using the Rap binding domain of RalGDS [75].

Chick Embryo Experimental Metastasis System

Fertilized White Leghorn eggs (McIntyre poultry and fertile eggs, Lakeside CA) were incubated in a rotary incubator at 38°C with 60% humidity. The chorioallantoic membrane was dropped at day 10 of incubation. Then 10^6 MDA-MB435 cells in 25 µl of serum-free DMEM were mixed with 25 µl of Matrigel (BD Biosciences) and inoculated onto the membrane. Following an additional 7 days of incubation, tumors growing on the membrane were excised, weighed, and prepared for histological analysis. Tumor cell colonization to the liver after intravenous injection into chick embryos was quantified by human specific *Alu*-real time PCR as described [76]. For microscopy, harvested tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Tissue sections were prepared at 4 µm and incubated with proteinase K (20 µg/ml) for antigen retrieval. After blocking, sections were incubated with primary antibody and, after washing with phosphate-buffered saline, with the relevant fluorescein (FITC)-conjugated secondary antibody. After washing, sections were incubated with auto-fluorescence eliminator reagent (EMD Millipore, San Diego, CA) and antibody binding was analyzed in a confocal microscope (FV1000; Olympus, Center Valley, PA).

To assess tumor cell extravasation into the chick liver, 3.0×10^5 MDA-MB435 cells labeled with tdTomato were injected into the chick embryo allantoic veins of day 12 eggs. Twenty-four hours later, 100 µl of FITC-conjugated lectin (*Lens culinaris* agglutinin, 500 µg/ml; Vector Laboratories, Burlingame, CA) were injected into the allantoic vein to label hepatic sinusoids. Livers were excised five minutes later and their surfaces were observed in a FV300 two-photon confocal microscope (Olympus, Center Valley, PA). Volocity software (PerkinElmer, Waltham, MA) was used to prepare three-dimensional images, and the number of tumor cells extravasated to the outside of the liver sinusoids was quantified.

Analysis of Hepatic Metastasis in the Mouse

C57Bl/6 mice were anesthetized with isoflurane and the spleen was exposed by a small incision in the left flank. Then 0.75×10^6 B16F10 mouse melanoma cells were injected into the spleen with a 30-gauge needle. Seven days later, livers were excised and imaged to assess the extent of macroscopic black tumors on the liver surface.

Analyses of Human Tumors

A human melanoma tissue array (ME804) containing 54 cases of primary melanomas and 26 cases of metastatic melanomas was obtained from US Biomax Inc (Rockville, MD). After staining with antibodies 9EG7 for activated β1, 4B7R for total β1, and melanoma marker MART-1 or epithelial marker cytokeratin, images were captured in an FV1000 confocal microscope and NanoZoomer 2.0HT (Hamamatsu, Shizuoka, Japan). Tumor-bearing areas positive for total β1 and activated β1 integrins were analyzed using Volocity or Image J software. All tissue array images were processed and analyzed identically.

Statistics

Mann-Whitney U test or Student's t-test (unpaired and two-tailed) was performed as indicated.

Supporting Information

Figure S1.
β1 integrin expression is required for hepatic colonization by tumor cells. (A) β1 integrin expression determined by flow cytometry. The expression levels of β1 integrin in MDA-MB435 cells infected with control lentivirus or lentivirus encoding β1 integrin shRNA were determined by binding of antibody TS2/16. Data represent mean fluorescence intensity (MFI) ± SEM (n = 10). (B) Box plot showing the number of control (n = 33) and β1 knock down cells (n = 37) that colonized chick embryo livers five days after intravenous tumor cell injection.
doi:10.1371/journal.pone.0046576.s001
(TIF)

Figure S2.
β3 integrin expression is not required for hepatic colonization by tumor cells. (A) β3 integrin expression determined by flow cytometry. Expression levels of β3 integrin in MDA-MB435 cells infected with lentivirus encoding control or β3 integrin shRNA were determined by binding of antibody SSA6. Data represent mean fluorescence intensity (MFI) ± SEM (n = 4). (B) Box plot showing the number of control (n = 20) and β3 knock down cells (n = 18) that colonized chick embryo livers five days after intravenous tumor cell injection.
doi:10.1371/journal.pone.0046576.s002
(TIF)

Figure S3.
Impaired adhesion of MDA-MB435 cells expressing β1-D130A. (A) Expression levels of β1 integrins were determined by flow cytometry. (B) β1-WT or β1-D130A MDA-MB435 cells were incubated in 96 well plates coated with the indicated concentrations of collagen or laminin for 60 minutes at 37°C. (n = 3). (C) Extrinsic integrin stimulation with 0.5 mM $MnCl_2$ does not induce cell adhesion of β1-D130A cells to collagen (1.25 µg/ml) or laminin (12.5 µg/ml) (n = 3). Data are expressed as cell adhesion normalized to total input of cells and represent means ± SEM.
doi:10.1371/journal.pone.0046576.s003
(TIF)

Figure S4.
Collagen receptor α2β1 promotes hepatic colonization by MDA-MB435 tumor cells. (A) Expression of the indicated α integrins before and after knock down of specific integrin α subunits was determined by flow cytometry. Data represent mean fluorescence intensity (MFI) ± SEM (α1 knock down: n = 5, α2 knock down: n = 6, α3 knock down: n = 9, α5 knock down: n = 5, α6 knock down: n = 4). (B) Box plot showing the number of MDA-MB435 cells in chick embryo livers five days after intravenous injection of tumor cells (Control: n = 92, α1 knock down: n = 52, α2 knock down: n = 52, α3 knock down: n = 42, α5 knock down: n = 38, α6 knock down: n = 35).
doi:10.1371/journal.pone.0046576.s004
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Figure S5.
Interaction of talin with β1 integrin is required for tumor cell adhesion. (A) Pull-down of talin from MDA-MB435 cell lysates by recombinant β1-WT or β1-I782A cytoplasmic tails. Talin was detected by western blotting using anti-talin antibody 8D4. (B) β1-WT and β1-I782A MDA-MB435 cells were incubated in 96 well plates coated with increasing concentrations of collagen or laminin for 60 minutes at 37°C and cell adhesion was analyzed. (n = 3). (C) Adhesion of β1-WT and β1-I782A MDA-MB435 cells to 1.25 µg/ml collagen or 12.5 µg/ml laminin. Where indicated, integrins were activated extrinsically with 0.5 mM $MnCl_2$ (n = 3± SEM).
doi:10.1371/journal.pone.0046576.s005
(TIF)

Figure S6.
Hepatic colonization of tumor cells 24 hours after intravenous injection into chick embryos. Box plot shows the number of β1-WT (n = 88) and β1-I782A cells (n = 75) present in the liver.
doi:10.1371/journal.pone.0046576.s006
(TIF)

Figure S7.
Talin expression is required for β1 integrin-mediated tumor cell adhesion. (A) Expression of talin in MDA-MB435 cells infected with lentivirus encoding control or either of three talin shRNAs was determined by Western blotting. (B) β1 integrin expression of control and talin knockdown MDA-MB435 cells was determined by flow cytometry. (C) Cell adhesion to collagen and laminin. Control and talin knockdown MDA-MB435 cells expressing β1-WT were incubated in 96 well plates coated with increasing concentrations of collagen or laminin for 60 minutes at 37°C. (n = 3). (D) Comparison of the effects of talin knock down on MDA-MB435 cells expressing either β1-WT or constitutively-active β1-L358A (n = 3± SEM).
doi:10.1371/journal.pone.0046576.s007
(TIF)

Figure S8.
Rap1GAP regulates β1 integrin-mediated tumor cell adhesion. (A) Pull-down assay for activated Rap1 in MDA-MB435 cells. Lysates from β1-WT and β1-L358A cells infected with GFP or GFP-Rap1GAP were incubated with GST-RalGDS and the binding of active GTP-Rap1 was analyzed by western blotting. MDA-MB435 cells expressing GFP-Rap1V12 and N17 served as positive and negative controls, respectively. (B) Effect of Rap1GAP overexpression on cell adhesion to collagen or laminin in cells expressing β1-WT or constitutively-active β1-L358A (n = 3± SEM).
doi:10.1371/journal.pone.0046576.s008
(TIF)

Figure S9.
Activated β1 integrin in formalin-fixed paraffin-embedded tumor cell specimens. 10^6 β1-WT cells, β1 integrin knockdown cells (β1 shRNA) and constitutively-active β1-L358A MDA-MB435 cells were resuspended in Matrigel and implanted onto the chorioallantoic membrane of day 10 chick embryos. After seven days of additional incubation, tumors were fixed in formalin and embedded into paraffin. Tumor sections were incubated with proteinase K for antigen retrieval and stained with antibodies for total β1 integrin (4B7R) and activated β1 integrin (9EG7).
doi:10.1371/journal.pone.0046576.s009
(TIF)

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Author Contributions

Conceived and designed the experiments: HK EID JPQ SJS. Performed the experiments: HK ZL JVM. Analyzed the data: HK ZL H-YW EID JAV JPQ SJS. Contributed reagents/materials/analysis tools: JAV. Wrote the paper: HK EID JAV JPQ SJS.

References

1. Nguyen DX, Bos PD, Massague J (2009) Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 9: 274–284. doi: 10.1038/nrc2622
View Article • PubMed/NCBI • Google Scholar

2. Fidler IJ (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 3: 453–458. doi: 10.1038/nrc1098
View Article • PubMed/NCBI • Google Scholar

3. Valastyan S, Weinberg RA (2011) Tumor metastasis: molecular insights and evolving paradigms. Cell 147: 275–292. doi: 10.1016/j.cell.2011.09.024
View Article • PubMed/NCBI • Google Scholar

4. Labelle M, Begum S, Hynes RO (2011) Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 20: 576–590. doi: 10.1016/j.ccr.2011.09.009
View Article • PubMed/NCBI • Google Scholar

5. Pawelek JM, Chakraborty AK (2008) Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis. Nat Rev Cancer 8: 377–386. doi: 10.1038/nrc2371
View Article • PubMed/NCBI • Google Scholar

6. Psaila B, Lyden D (2009) The metastatic niche: adapting the foreign soil. Nat Rev Cancer 9: 285–293. doi: 10.1038/nrc2621
View Article • PubMed/NCBI • Google Scholar

7. Duda DG, Duyverman AM, Kohno M, Snuderl M, Steller EJ, et al. (2010) Malignant cells facilitate lung metastasis by bringing their own soil. Proc Natl Acad Sci U S A 107: 21677–21682. doi: 10.1073/pnas.1016234107
View Article • PubMed/NCBI • Google Scholar

8. Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, et al. (2009) Tumor self-seeding by circulating cancer cells. Cell 139: 1315–1326. doi: 10.1016/j.cell.2009.11.025
View Article • PubMed/NCBI • Google Scholar

9. Jeanes A, Gottardi CJ, Yap AS (2008) Cadherins and cancer: how does cadherin dysfunction promote tumor progression? Oncogene 27: 6920–6929. doi: 10.1038/onc.2008.343
View Article • PubMed/NCBI • Google Scholar

10. Desgrosellier JS, Cheresh DA (2010) Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 10: 9–22. doi: 10.1038/nrc2748
View Article • PubMed/NCBI • Google Scholar

11. Yilmaz M, Christofori G (2009) EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 28: 15–33. doi: 10.1007/s10555-008-9169-0
View Article • PubMed/NCBI • Google Scholar

12. Guo W, Giancotti FG (2004) Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 5: 816–826. doi: 10.1038/nrm1490
View Article • PubMed/NCBI • Google Scholar

13. Brakebusch C, Fassler R (2005) β1 integrin function in vivo: adhesion, migration and more. Cancer Metastasis Rev 24: 403–411. doi: 10.1007/s10555-005-5132-5
View Article • PubMed/NCBI • Google Scholar

14.

Yoshimura K, Meckel KF, Laird LS, Chia CY, Park JJ, et al. (2009) Integrin α2 mediates selective metastasis to the liver. Cancer Res 69: 7320–7328. doi: 10.1158/0008-5472.can-09-0315
View Article • PubMed/NCBI • Google Scholar

15. Morini M, Mottolese M, Ferrari N, Ghiorzo F, Buglioni S, et al. (2000) The α3β1 integrin is associated with mammary carcinoma cell metastasis, invasion, and gelatinase B (MMP-9) activity. Int J Cancer 87: 336–342. doi: 10.1002/1097-0215(20000801)87:3<336::aid-ijc5>3.0.co;2-3
View Article • PubMed/NCBI • Google Scholar

16. Natali PG, Nicotra MR, Bartolazzi A, Cavaliere R, Bigotti A (1993) Integrin expression in cutaneous malignant melanoma: association of the α3β1 heterodimer with tumor progression. Int J Cancer 54: 68–72. doi: 10.1002/ijc.2910540112
View Article • PubMed/NCBI • Google Scholar

17. Danen EH, Ten Berge PJ, Van Muijen GN, Van't Hof-Grootenboer AE, Brocker EB, et al. (1994) Emergence of α5β1 fibronectin- and αVβ3 vitronectin-receptor expression in melanocytic tumour progression. Histopathology 24: 249–256. doi: 10.1111/j.1365-2559.1994.tb00517.x
View Article • PubMed/NCBI • Google Scholar

18. Adachi M, Taki T, Higashiyama M, Kohno N, Inufusa H, et al. (2000) Significance of integrin α5 gene expression as a prognostic factor in node-negative non-small cell lung cancer. Clin Cancer Res 6: 96–101.
View Article • PubMed/NCBI • Google Scholar

19. Wewer UM, Shaw LM, Albrechtsen R, Mercurio AM (1997) The integrin α6β1 promotes the survival of metastatic human breast carcinoma cells in mice. Am J Pathol 151: 1191–1198.
View Article • PubMed/NCBI • Google Scholar

20. Carbonell WS, Ansorge O, Sibson N, Muschel R (2009) The vascular basement membrane as "soil" in brain metastasis. PLoS One 4: e5857.
View Article • PubMed/NCBI • Google Scholar

21. Shibue T, Weinberg RA (2009) Integrin β1-focal adhesion kinase signaling directs the proliferation of metastatic cancer cells disseminated in the lungs. Proc Natl Acad Sci U S A 106: 10290–10295. doi: 10.1073/pnas.0904227106
View Article • PubMed/NCBI • Google Scholar

22. Huck L, Pontier SM, Zuo DM, Muller WJ (2010) β1-integrin is dispensable for the induction of ErbB2 mammary tumors but plays a critical role in the metastatic phase of tumor progression. Proc Natl Acad Sci U S A 107: 15559–15564. doi: 10.1073/pnas.1003034107
View Article • PubMed/NCBI • Google Scholar

23. Grzesiak JJ, Tran Cao HS, Burton DW, Kaushal S, Vargas F, et al. (2011) Knockdown of the β1 integrin subunit reduces primary tumor growth and inhibits pancreatic cancer metastasis. Int J Cancer 129: 2905–2915. doi: 10.1002/ijc.25942
View Article • PubMed/NCBI • Google Scholar

24. Yao ES, Zhang H, Chen YY, Lee B, Chew K, et al. (2007) Increased β1 integrin is associated with decreased survival in invasive breast cancer. Cancer Res 67: 659–664. doi: 10.1158/0008-5472.can-06-2768
View Article • PubMed/NCBI • Google Scholar

25. Oshita F, Kameda Y, Hamanaka N, Saito H, Yamada K, et al. (2004) High expression of integrin β1 and p53 is a greater poor prognostic factor than clinical stage in small-cell lung cancer. Am J Clin Oncol 27: 215–219. doi: 10.1097/01.coc.0000054894.64867.80
View Article • PubMed/NCBI • Google Scholar

26. Han JY, Kim HS, Lee SH, Park WS, Lee JY, et al. (2003) Immunohistochemical expression of integrins and extracellular matrix proteins in non-small cell lung cancer: correlation with lymph node metastasis. Lung Cancer 41: 65–70. doi: 10.1016/s0169-5002(03)00146-6
View Article • PubMed/NCBI • Google Scholar

27. Dingemans AM, van den Boogaart V, Vosse BA, van Suylen RJ, Griffioen AW, et al. (2010) Integrin expression profiling identifies integrin α5 and β1 as prognostic factors in early stage non-small cell lung cancer. Mol Cancer 9: 152. doi: 10.1186/1476-4598-9-152
View Article • PubMed/NCBI • Google Scholar

28. Bottger TC, Maschek H, Lobo M, Gottwohl RG, Brenner W, et al. (1999) Prognostic value of immunohistochemical expression of β1 integrin in pancreatic carcinoma. Oncology 56: 308–313. doi: 10.1159/000011984
View Article • PubMed/NCBI • Google Scholar

29. Nikkola J, Vihinen P, Vlaykova T, Hahka-Kemppinen M, Heino J, et al. (2004) Integrin chains β1 and αV as prognostic factors in human metastatic melanoma. Melanoma Res 14: 29–37. doi: 10.1097/00008390-200402000-00005
View Article • PubMed/NCBI • Google Scholar

30. Vuoristo M, Vihinen P, Vlaykova T, Nylund C, Heino J, et al. (2007) Increased gene expression levels of collagen receptor integrins are associated with decreased survival parameters in patients with advanced melanoma. Melanoma Res 17: 215–223. doi: 10.1097/cmr.0b013e328270b935
View Article • PubMed/NCBI • Google Scholar

31. Ramirez NE, Zhang Z, Madamanchi A, Boyd KL, O'Rear LD, et al. (2011) The α2β1 integrin is a metastasis suppressor in mouse models and human cancer. J Clin Invest 121: 226–237. doi: 10.1172/jci42328
View Article • PubMed/NCBI • Google Scholar

32. Zutter MM, Santoro SA, Staatz WD, Tsung YL (1995) Re-expression of the α2β1 integrin abrogates the malignant phenotype of breast carcinoma cells. Proc Natl Acad Sci U S A 92: 7411–7415. doi: 10.1073/pnas.92.16.7411
View Article • PubMed/NCBI • Google Scholar

33. Wang H, Fu W, Im JH, Zhou Z, Santoro SA, et al. (2004) Tumor cell α3β1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. J Cell Biol 164: 935–941. doi: 10.1083/jcb.200309112
View Article • PubMed/NCBI • Google Scholar

34. Barkan D, Chambers AF (2011) β1-integrin: a potential therapeutic target in the battle against cancer recurrence. Clin Cancer Res 17: 7219–7223. doi: 10.1158/1078-0432.ccr-11-0642
View Article • PubMed/NCBI • Google Scholar

35. Shattil SJ, Kim C, Ginsberg MH (2010) The final steps of integrin activation: the end game. Nat Rev Mol Cell Biol 11: 288–300. doi: 10.1038/nrm2871
View Article • PubMed/NCBI • Google Scholar

36. Hogg N, Patzak I, Willenbrock F (2011) The insider's guide to leukocyte integrin signalling and function. Nat Rev Immunol 11: 416–426. doi: 10.1038/nri2986

View Article • PubMed/NCBI • Google Scholar

37. Petrich BG (2009) Talin-dependent integrin signalling in vivo. Thromb Haemost 101: 1020–1024. doi: 10.1160/th08-08-0553
View Article • PubMed/NCBI • Google Scholar

38. Petrich BG, Fogelstrand P, Partridge AW, Yousefi N, Ablooglu AJ, et al. (2007) The antithrombotic potential of selective blockade of talin-dependent integrin αIIbβ3 (platelet GPIIb-IIIa) activation. J Clin Invest 117: 2250–2259. doi: 10.1172/jci31024
View Article • PubMed/NCBI • Google Scholar

39. Petrich BG, Marchese P, Ruggeri ZM, Spiess S, Weichert RA, et al. (2007) Talin is required for integrin-mediated platelet function in hemostasis and thrombosis. J Exp Med 204: 3103–3111. doi: 10.1084/jem.20071800
View Article • PubMed/NCBI • Google Scholar

40. Nieswandt B, Moser M, Pleines I, Varga-Szabo D, Monkley S, et al. (2007) Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo. J Exp Med 204: 3113–3118. doi: 10.1084/jem.20071827
View Article • PubMed/NCBI • Google Scholar

41. Jung SM, Moroi M (2000) Signal-transducing mechanisms involved in activation of the platelet collagen receptor integrin α2β1. J Biol Chem 275: 8016–8026. doi: 10.1074/jbc.275.11.8016
View Article • PubMed/NCBI • Google Scholar

42. Desgrosellier JS, Barnes LA, Shields DJ, Huang M, Lau SK, et al. (2009) An integrin αVβ3-c-Src oncogenic unit promotes anchorage-independence and tumor progression. Nat Med 15: 1163–1169. doi: 10.1038/nm.2009
View Article • PubMed/NCBI • Google Scholar

43. Takada Y, Ylanne J, Mandelman D, Puzon W, Ginsberg MH (1992) A point mutation of integrin β1 subunit blocks binding of α5β1 to fibronectin and invasin but not recruitment to adhesion plaques. J Cell Biol 119: 913–921. doi: 10.1083/jcb.119.4.913
View Article • PubMed/NCBI • Google Scholar

44. Bouaouina M, Lad Y, Calderwood DA (2008) The N-terminal domains of talin cooperate with the phosphotyrosine binding-like domain to activate β1 and β3 integrins. J Biol Chem 283: 6118–6125. doi: 10.1074/jbc.m709527200
View Article • PubMed/NCBI • Google Scholar

45. Calderwood DA, Zent R, Grant R, Rees DJ, Hynes RO, et al. (1999) The Talin head domain binds to integrin β subunit cytoplasmic tails and regulates integrin activation. J Biol Chem 274: 28071–28074. doi: 10.1074/jbc.274.40.28071
View Article • PubMed/NCBI • Google Scholar

46. Wegener KL, Partridge AW, Han J, Pickford AR, Liddington RC, et al. (2007) Structural basis of integrin activation by talin. Cell 128: 171–182. doi: 10.1016/j.cell.2006.10.048
View Article • PubMed/NCBI • Google Scholar

47. Zheng H, Gao L, Feng Y, Yuan L, Zhao H, et al. (2009) Down-regulation of Rap1GAP via promoter hypermethylation promotes melanoma cell proliferation, survival, and migration. Cancer Res 69: 449–457. doi: 10.1158/0008-5472.can-08-2399
View Article • PubMed/NCBI • Google Scholar

48. Bos JL (2005) Linking Rap to cell adhesion. Curr Opin Cell Biol 17: 123–128. doi: 10.1016/j.ceb.2005.02.009
View Article • PubMed/NCBI • Google Scholar

49. Lenter M, Uhlig H, Hamann A, Jeno P, Imhof B, et al. (1993) A monoclonal antibody against an activation epitope on mouse integrin chain beta 1 blocks adhesion of lymphocytes to the endothelial integrin α6β1. Proc Natl Acad Sci U S A 90: 9051–9055. doi: 10.1073/pnas.90.19.9051
View Article • PubMed/NCBI • Google Scholar

50. Bazzoni G, Shih DT, Buck CA, Hemler ME (1995) Monoclonal antibody 9EG7 defines a novel beta 1 integrin epitope induced by soluble ligand and manganese, but inhibited by calcium. J Biol Chem 270: 25570–25577. doi: 10.1074/jbc.270.43.25570
View Article • PubMed/NCBI • Google Scholar

51. Felding-Habermann B, O'Toole TE, Smith JW, Fransvea E, Ruggeri ZM, et al. (2001) Integrin activation controls metastasis in human breast cancer. Proc Natl Acad Sci U S A 98: 1853–1858. doi: 10.1073/pnas.98.4.1853
View Article • PubMed/NCBI • Google Scholar

52. Lonsdorf AS, Kramer BF, Fahrleitner M, Schonberger T, Gnerlich S, et al. (2012) Engagement of αIIbβ3 (GPIIb/IIIa) with αVβ3 integrin mediates interaction of melanoma cells with platelets: a connection to hematogenous metastasis. J Biol Chem 287: 2168–2178. doi: 10.1074/jbc.m111.269811
View Article • PubMed/NCBI • Google Scholar

53. Nieswandt B, Hafner M, Echtenacher B, Mannel DN (1999) Lysis of tumor cells by natural killer cells in mice is impeded by platelets. CancerRes 59: 1295–1300.
View Article • PubMed/NCBI • Google Scholar

54. Eto K, Murphy R, Kerrigan SW, Bertoni A, Stuhlmann H, et al. (2002) Megakaryocytes derived from embryonic stem cells implicate CalDAG-GEFI in integrin signaling. Proc Natl Acad Sci U S A 99: 12819–12824. doi: 10.1073/pnas.202380099
View Article • PubMed/NCBI • Google Scholar

55. Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, et al. (2003) Talin binding to integrin β tails: a final common step in integrin activation. Science 302: 103–106. doi: 10.1126/science.1086652
View Article • PubMed/NCBI • Google Scholar

56. Hernandez-Varas P, Colo GP, Bartolome RA, Paterson A, Medrano-Fernandez I, et al. (2011) Rap1-GTP-interacting adaptor molecule (RIAM) protein controls invasion and growth of melanoma cells. J Biol Chem 286: 18492–18504. doi: 10.1074/jbc.m110.189811
View Article • PubMed/NCBI • Google Scholar

57. Freeman SA, McLeod SJ, Dukowski J, Austin P, Lee CC, et al. (2010) Preventing the activation or cycling of the Rap1 GTPase alters adhesion and cytoskeletal dynamics and blocks metastatic melanoma cell extravasation into the lungs. Cancer Res 70: 4590–4601. doi: 10.1158/0008-5472.can-09-3414
View Article • PubMed/NCBI • Google Scholar

58. Sakamoto S, McCann RO, Dhir R, Kyprianou N (2010) Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance. Cancer Res 70: 1885–1895. doi: 10.1158/0008-5472.can-09-2833
View Article • PubMed/NCBI • Google Scholar

59. Shen Z, Ye Y, Dong L, Vainionpaa S, Mustonen H, et al. (2012) Kindlin-2: a novel adhesion protein related to tumor invasion, lymph node metastasis, and patient outcome in gastric cancer. Am J Surg 203: 222–229. doi: 10.1016/j.amjsurg.2011.06.050
View Article • PubMed/NCBI • Google Scholar

60. Sin S, Bonin F, Petit V, Meseure D, Lallemand F, et al. (2011) Role of the focal adhesion protein kindlin-1 in breast cancer growth and lung metastasis. J Natl Cancer Inst 103: 1323–1337. doi: 10.1093/jnci/djr290
View Article • PubMed/NCBI • Google Scholar

61. An Z, Dobra K, Lock JG, Stromblad S, Hjerpe A, et al. (2010) Kindlin-2 is expressed in malignant mesothelioma and is required for tumor cell adhesion and migration. Internat Cancer 127: 1999–2008. doi: 10.1002/ijc.25223
View Article • PubMed/NCBI • Google Scholar

62. Plow EF, Qin J, Byzova T (2009) Kindling the flame of integrin activation and function with kindlins. Curr Opin Hematol 16: 323–328. doi: 10.1097/moh.0b013e32832ea389
View Article • PubMed/NCBI • Google Scholar

63. Moser M, Legate KR, Zent R, Fassler R (2009) The tail of integrins, talin, and kindlins. Science 324: 895–899. doi: 10.1126/science.1163865
View Article • PubMed/NCBI • Google Scholar

64. Rantala JK, Pouwels J, Pellinen T, Veltel S, Laasola P, et al. (2011) SHARPIN is an endogenous inhibitor of β1-integrin activation. Nat Cell Biol 13: 1315–1324. doi: 10.1038/ncb2340
View Article • PubMed/NCBI • Google Scholar

65. Nevo J, Mai A, Tuomi S, Pellinen T, Pentikainen OT, et al. (2010) Mammary-derived growth inhibitor (MDGI) interacts with integrin α-subunits and suppresses integrin activity and invasion. Oncogene 29: 6452–6463. doi: 10.1038/onc.2010.376
View Article • PubMed/NCBI • Google Scholar

66. Pellinen T, Rantala JK, Arjonen A, Mpindi JP, Kallioniemi O, et al. (2012) A functional genetic screen reveals new regulators of β1-integrin activity. J Cell Sci 125: 649–661. doi: 10.1242/jcs.090704
View Article • PubMed/NCBI • Google Scholar

67. Zhang X, Jiang G, Cai Y, Monkley SJ, Critchley DR, et al. (2008) Talin depletion reveals independence of initial cell spreading from integrin activation and traction. Nat Cell Biol 10: 1062–1068. doi: 10.1038/ncb1765
View Article • PubMed/NCBI • Google Scholar

68. Qin XF, An DS, Chen IS, Baltimore D (2003) Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5. Proc Natl Acad Sci U S A 100: 183–188. doi: 10.1073/pnas.232688199
View Article • PubMed/NCBI • Google Scholar

69. Campbell RE, Tour O, Palmer AE, Steinbach PA, Baird GS, et al. (2002) A monomeric red fluorescent protein. Proc Natl Acad Sci U S A 99: 7877–7882. doi: 10.1073/pnas.082243699
View Article • PubMed/NCBI • Google Scholar

70. Stoletov K, Kato H, Zardouzian E, Kelber J, Yang J, et al. (2010) Visualizing extravasation dynamics of metastatic tumor cells. J Cell Sci 123: 2332–2341. doi: 10.1242/jcs.069443
View Article • PubMed/NCBI • Google Scholar

71. Mould AP, Barton SJ, Askari JA, McEwan PA, Buckley PA, et al. (2003) Conformational changes in the integrin βA domain provide a mechanism for signal transduction via hybrid domain movement. J Biol Chem 278: 17028–17035. doi: 10.1074/jbc.m213139200
View Article • PubMed/NCBI • Google Scholar

72. Watanabe N, Bodin L, Pandey M, Krause M, Coughlin S, et al. (2008) Mechanisms and consequences of agonist-induced talin recruitment to platelet integrin αIIbβ3. J Cell Biol 181: 1211–1222. doi: 10.1083/jcb.200803094
View Article • PubMed/NCBI • Google Scholar

73. Sims PJ, Ginsberg MH, Plow EF, Shattil SJ (1991) Effect of platelet activation on the conformation of the plasma membrane glycoprotein IIb-IIIa complex. J Biol Chem 266: 7345–7352.
View Article • PubMed/NCBI • Google Scholar

74. Arias-Salgado EG, Lizano S, Shattil SJ, Ginsberg MH (2005) Specification of the direction of adhesive signaling by the integrin β cytoplasmic domain. J Biol Chem 280: 29699–29707. doi: 10.1074/jbc.m503508200
View Article • PubMed/NCBI • Google Scholar

75. Bertoni A, Tadokoro S, Eto K, Pampori N, Parise LV, et al. (2002) Relationships between Rap1b, affinity modulation of integrin αIIbβ3, and the actin cytoskeleton. J Biol Chem 277: 25715–25721. doi: 10.1074/jbc.m202791200
View Article • PubMed/NCBI • Google Scholar

76. Deryugina EI, Zijlstra A, Partridge JJ, Kupriyanova TA, Madsen MA, et al. (2005) Unexpected effect of matrix metalloproteinase down-regulation on vascular intravasation and metastasis of human fibrosarcoma cells selected in vivo for high rates of dissemination. Cancer Res 65: 10959–10969. doi: 10.1158/0008-5472.can-05-2228
View Article • PubMed/NCBI • Google Scholar

Поз.7.26.
Превод от английски език

Antibodies-Online

Oxidized Low Density Lipoprotein (Lectin-Like) Receptor 1 (OLRl) (N-Term) antibody

Антиген:	Oxidized Low Density Lipoprotein (Lectin-Like) Receptor 1 (OLRl)
Синоними:	PLOX-l, CLEC8A, LOXl, LOXIN, SCAREl, SLOXl, LOX-I, SR-EI, Scarel, Oldlrl, Oldrl, Olrl, OLRI
Епитоп	Амино края
Реактивност:	Човек, Мишка, Плъх
Гостоприемник:	Заек
Клоналност:	Поликлонално
Приложение:	Имунохистохимия, Имуноблот
Каталожен номер:	ABIN223339
Количество:	100 µg
Концентрация	0.5 mg/ml
Наличност:	.....

Информация за продукта

Имуноген:	Синтетичен пептид близо до аминокрая на човешки Lox-
Изотип:	IgG
Пречистване:	Афинитетно пречистване
Общи данни:	----------

Детайли на приложение

Бележки за прилагане:	Имуноблот (0.5-4 µg/mL). Открива про- (приблизително 50 kDa) и зрялата (приблизително 30 kDa) форми на Lox-l в проби от човешки, миши и плъши органи. Други приложения не са определяни
Ограничения:	Само за изследователски цели

Манипулации

Формат:	Течен
Буфер:	PBS pH 7.2, съдържащ 30% глицерол, 0.5% BSA и 0.01% тримерозал
Консерванти:	Тримерозал
Съхранение:	-20 °C/-80 °C За по-продължително съхранение, разфасовайте и замразете на -70 °C
Срок на годност	12 месеца

Превод от английски език

Лого на Santa Cruz Biotechnology, Inc.

TLR4 (H-80): sc-10741

Обща информация: ----------

Хромозомна локализация: Генетичен локус: TLR4 (човек) – 9q33.1; Tlr4 (мишка) – 4 C1

Източник: TLR4 (H-80) е заешко поликлонално антитяло, получено срещу аминокиселини 242-321 на TLR4 от човешки произход.

Продукт: Всяка виалка съдържа 200 µg IgG в 1.0 мл. PBS с <0.1% натриев азид и 0.1% желатин.

Приложения: TLR4 (H-80) се препоръчва за доказване на TLR4 от човешки и в по-малка степен от миши и плъши произход посредством Имуноблот (начално разреждане 1:200, диапазон на разреждане 1:100-1:1000), Имунопреципитация [1-2 µg на 100-500 µg общ протеин (1 ml клетъчен лизат)], Имунофлуоресценция (начално разреждане 1:50, диапазон на разреждане 1:50-1:500) и твърдо-фазова ELISA (начално разреждане 1:30, диапазон на разреждане 1:30-1:3000).

Подходящо за използване като контролно антитяло за TLR4 siRNA (h): sc-40260, TLR4 siRNA (m): sc-40261, TLR4 siRNA (r): sc-156001, TLR4 shRNA Plasmid (h): sc-40260-SH, TLR4 shRNA Plasmid (m): sc-40261-SH, TLR4 shRNA Plasmid (r): sc-156001-SH, TLR4 shRNA (h) Lentiviral Particles: sc-40260-V, TLR4 shRNA (m) Lentiviral Particles: sc-40261-V и TLR4 shRNA (r) Lentiviral Particles: sc-156001-V.

Молекулна маса на TLR4: 95 kDa.

Молекулна маса на глшкозилиран TLR4: 120 kDa.

Положителна контрола: HEL 92.1.7 клетъчен лизат: sc-2270, THP-1 клетъчен лизат: sc-2238 или HL-60 тотален клетъчен лизат: sc-2209

Съхранение: Съхранявайте при 4° C, **ДА НЕ СЕ ЗАМРАЗЯВА**. Стабилен за една година от датата на доставка. Не е опасен. Не изисква MSDS.

Изследователска употреба: Само за изследователски цели, а не за диагностични процедури