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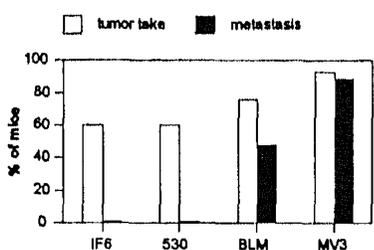
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**Mechanisms of melanoma cell adhesion to fibronectin.**

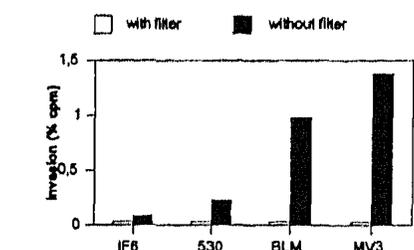
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Recent observations have shown that besides soluble factors, also the extracellular matrix influences gene expression and controls proliferation and differentiation of cells. The integrin family of cell surface receptors is involved in the transduction of signals from the extracellular matrix to the nucleus [1]. This function, together with their role in cell adhesion and migration has initiated much research on the role of integrins in cancer. For human melanoma, changes in the expression of  $\alpha 3\beta 1$ ,  $\alpha 4\beta 1$ ,  $\alpha 5\beta 1$ , and  $\alpha v\beta 3$  have been found to correlate with tumor progression [reviewed in 2]. All these integrins can bind fibronectin (Fn).

We have investigated the mechanism of adhesion to Fn of human melanocytes (mct) and four human melanoma cell lines with different metastatic capacities in nude mice. All 4 melanoma cell lines investigated were tumorigenic but IF6 and 530 were non-metastatic whereas BLM and MV3 were highly metastatic (fig. 1). In line with these findings, only BLM and MV3 were highly invasive through a



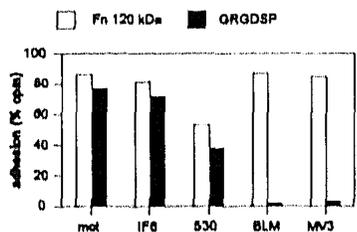
**Fig 1: Metastatic capacity of human melanoma cell lines.** Cells were inoculated s.c. into nude mice and lungs were inspected for nodules after 2-3 months.



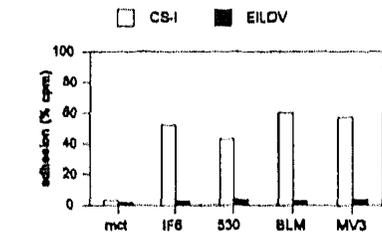
**Fig 2: Invasion of human melanoma cells.** Radiolabeled cells were allowed to invade an amniotic basement membrane. A filter was used to correct for leakage of radioactive debris.

human amniotic basement membrane (fig. 2).

Mct and all 4 melanoma cell lines adhere to Fn [3]. Cell adhesion can be promoted by several regions of the Fn molecule including the central cell binding domain, the HepII domain, and the CS-I region [reviewed in 4]. To determine the regions in Fn involved in attachment of the various cell lines, we performed adhesion assays to a) a 120 kDa Fn fragment containing the central cell binding domain but not HepII or CS-I, b) a peptide containing the RGD recognition site from the central cell binding domain, c) a CS-I fragment, and d) a peptide containing the LDV recognition site from CS-I. Mct and



**Fig 3: Adhesion to the central cell binding domain.** Radiolabeled cells were allowed to adhere for 30 min at 37°C to wells coated with a 120 kDa Fn fragment or with a GRGDSP peptide coupled to BSA. Adherent cells were lysed and radioactivity was measured.



**Fig 4: Adhesion to the CS-I domain.** Radiolabeled cells were allowed to adhere to wells coated with a CS-I peptide coupled to Ig or with a EILDV peptide coupled to BSA. Adherent cells were lysed and radioactivity was measured.

melanoma cells adhered to the 120 kDa fragment but only mct, IF6, and 530 adhered to GRGDSP (fig. 3). Melanoma cells but not mct adhered to CS-I whereas non of the cell lines adhered to EILDV (fig. 4). As shown in table 1, in contrast to the melanoma cell lines, mct did not express  $\alpha 4\beta 1$ . This explains the lack of binding of mct to CS-I since  $\alpha 4\beta 1$  is the receptor for that region in Fn. In line with the finding that all cells adhered to the central cell binding domain, they all expressed  $\alpha 3\beta 1$  and  $\alpha 5\beta 1$ . In addition, mct, IF6, and 530, but not BLM and MV3 expressed  $\alpha v\beta 3$ . These are all receptors for RGD in

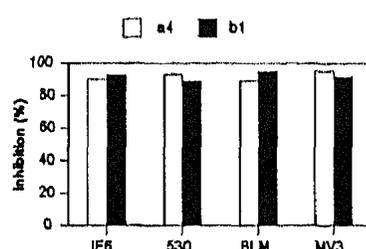
the central cell binding domain. The fact that only mct, IF6, and 530 adhered to the GRGDSP peptide, was likely to be due to lack of  $\alpha v\beta 3$  on BLM and MV3.

We performed antibody inhibition studies to identify integrins involved in adhesion to the different adhesive ligands. Adhesion of all melanoma cell lines to CS-I was blocked by mAbs to  $\alpha 4\beta 1$  (fig. 5). P1B5 anti- $\alpha 3$  had no effect in any of the assays (not shown). Adhesion of mct, IF6, and 530 to the central cell binding domain was partially inhibited by  $\alpha 5$  or  $\alpha v\beta 3$  mAbs and adhesion was completely blocked by a

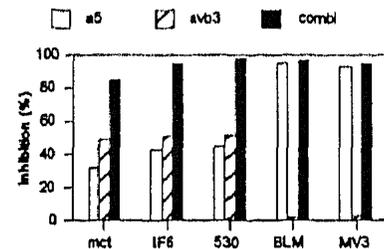
**Table 1: Expression of Fn binding integrins on melanocytes and melanoma cell lines.**

	mct	IF6	530	BLM	MV3
control	5*	4	6	5	3
$\alpha 3$	31	45	29	51	57
$\alpha 4$	4	23	19	36	39
$\alpha 5$	41	45	33	57	55
$\beta 1$	81	87	66	113	127
$\alpha v$	32	29	18	16	15
$\alpha v\beta 3$	34	32	23	6	3

\*mean fluorescence



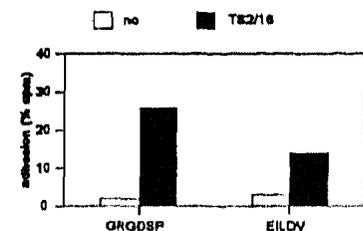
**Fig 5: Inhibition of adhesion to CS-I.** Radiolabeled cells were incubated with HP2/1 anti- $\alpha 4$  or 4B4 anti- $\beta 1$  30 min prior to addition to CS-I coated wells.



**Fig 6: Inhibition of adhesion to the central cell binding domain.** NK1-SAM1 anti- $\alpha 5$ , LM609 anti- $\alpha v\beta 3$ , or both were used as in fig. 5 to inhibit adhesion to a Fn 120 kDa fragment.

combination of these mAbs (fig. 6). In contrast, for total inhibition of adhesion of BLM and MV3,  $\alpha 5$  mAbs were sufficient (fig. 6).

Lack of adhesion to GRGDSP and EILDV by cells that adhere to the central cell binding domain through  $\alpha 5\beta 1$  and to CS-I through  $\alpha 4\beta 1$ , may be due to expression of  $\beta 1$ -integrins in a partially active state. Therefore we treated the cells with TS2/16 anti- $\beta 1$  mAbs that induce a high affinity state of the  $\beta 1$  integrins. TS2/16 induced adhesion of BLM (fig. 7) and MV3 (not shown) to GRGDSP and EILDV, and this adhesion was fully blocked by  $\alpha 5$  and  $\alpha 4$  mAbs respectively (not shown). No induction of binding to EILDV was found for mct, IF6, or 530.



**Fig 7: Induction of adhesion to GRGDSP and EILDV by TS2/16.** BLM cells were incubated with TS2/16 30 min prior to addition to the wells.

In conclusion, mct and all melanoma cell lines adhere to Fn, but differential expression of  $\alpha 4\beta 1$ ,  $\alpha 5\beta 1$ , and  $\alpha v\beta 3$  leads to different binding mechanisms. For binding their minimal recognition sequence,  $\alpha 4\beta 1$ ,  $\alpha 5\beta 1$ , but not  $\alpha v\beta 3$  require an activating signal. Such signals may come from other sites in the Fn molecule and can be mimicked by TS2/16. Requirement of the reported synergy site for  $\alpha 5\beta 1$  binding to RGD [5] is indeed modulated by TS2/16 (not shown).

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