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 α3β1, α4β1, α5β1, and αvβ3 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 S30 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 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 melanoma cells adhered to the 120 kDa fragment but only mct, IF6, and S30 adhered to GRGDSP (fig. 3). Melanoma cells but not mct adhered to CS-I whereas none 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 α4β1. This explains the lack of binding of mct to CS-I since α4β1 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 α3β1 and α5β1. In addition, mct, IF6, and S30, but not BLM and MV3 expressed αvβ3. These are all receptors for RGD in combination of these mAbs (fig. 6). In contrast, for total inhibition of adhesion of BLM and MV3, α5 mAbs were sufficient (fig. 6).

Lack of adhesion to GRGDSP and EILDV by cells that adhere to the central cell binding domain through α5β1 and to CS-I through α4β1, may be due to expression of β1-integrins in a partially active state. Therefore we treated the cells with TS2/16 anti-β1 mAbs that induce a high affinity state of the β1 integrins. TS2/16 induced adhesion of BLM (fig. 7) and MV3 (not shown) to GRGDSP and EILDV, and this adhesion was fully blocked by α5 and α4 mAbs respectively (not shown). No induction of binding to EILDV was found for mct, IF6, or S30.

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

<table>
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<tr>
<th></th>
<th>mct</th>
<th>IF6</th>
<th>S30</th>
<th>BLM</th>
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In conclusion, mct and all melanoma cell lines adhere to Fn, but differential expression of α4β1, α5β1, and αvβ3 leads to different binding mechanisms. For binding their minimal recognition sequence, α4β1, α5β1, but not αvβ3 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 αvβ3 binding to RGD [5] is indeed modulated by TS2/16 (not shown).

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