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S4.11

Unsialylated PNA Binding Sites in Human Melanoma: A New Marker of Metastatic Potential

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Cell surface glycoconjugates influence the metastatic behaviour of tumor cells. Using a recently developed model of human tumor spontaneous metastasis (1), we examined changes in cell surface carbohydrate moieties in melanoma cells associated with metastasis. Lectin binding patterns of seven human melanoma clones and variants, selected from the same parental cell line (2), were compared by flow cytometry and Scatchard analysis. Human melanoma clones/variants with high (HM) and low metastatic (LM) potential could be distinguished by their PNA binding, but not by their WGA, UEA I and SBA binding. LM clones/variants proved to be made up from a single cell population, poorly labeled by PNA (2.2 10^6 sites/cell). In contrast, HM clones/variants were constituted by two cellular subpopulations exhibiting a moderate (2.6 – 3.7 10^6 sites/cell) and a high PNA staining (17.7 – 18.8 10^6 sites/cell). Using an EPICS V cytometer, these two subpopulations were sorted and tested for their metastatic abilities. Cells with high PNA binding generate a higher frequency of metastases than cells with moderate PNA binding. Following treatment with *Vibrio Cholerae* neuraminidase, all cells from all variants and clones were brightly labeled by PNA, collecting in a single peak with similar fluorescence intensity. We have identified 2 major PNA reactive glycoproteins of 110 and 140 kDa, expressed only in HM cells, which became strongly labeled in the LM cells only after neuraminidase treatment. These results suggest that the expression of terminal unsialylated Galβ1-3GalNAc structure, specifically positioned on two glycoproteins 140 kDa and 110 kDa, play a key role in the metastatic potential of human melanoma cells. These carbohydrate alterations detected in cells *in vitro* are also expressed by tumor cells *in vivo*. In 37 specimens tested, PNA appears to react selectively with invasive tumors, since none of the four early primary melanomas (Clark I-II) reacted while 10/16 late primary melanomas (Clark III-V) and 3/5 melanoma metastases were reactive. Only 1/12 benign nevi bound PNA. Our data gave evidence that the non sialylation of terminal Galβ1-3GalNAc carbohydrate may be markers of invasive melanoma, applicable on an individual patient basis.

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S4.12

Polylactosaminoglycans of Lysosomal Membrane Glycoproteins Lamp-1 and Lamp-2 in Human Milk

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In a previous study (1) we have shown that lamp-1 and lamp-2 are secreted into milk from mothers of preterm infants. Here, we investigated whether lamp-1 and lamp-2 are major carriers of polylactosaminoglycans in human milk. Immunological studies and lectin experiments have been performed by Western Blotting of casein fractions, whey and whey fractions after Superdex chromatography. Antibodies and lectins used were: mouse monoclonal anti-lamp-1 (BB6) and anti-lamp-2 (CD3) and the lectins of Datura stramonium, Phaseolus vulgaris-L, Lycopersicon esculentum, Lotus tetragonolobus, Sambucus nigra and Maackia amurensis. We showed that a) lamp-1 and lamp-2 are secreted in significant amounts in human milk; b) both antibodies reacted with components in the whey fractions; c) anti-lamp-1 and anti-lamp-2 bound to three bands (MW >200 kD, ≈ 130 kD, ≈ 80 kD); d) Lycopersicon esculentum and Lotus tetragonolobus reacted only with a band of MW ≈ 80 kD; e) Paseolus vulgaris I lectin resulted in an almost identical pattern as compared to anti-lamp-1 and anti-lamp-2. The results show that human milk does contain lysosomal membrane glycoproteins with a different degree of glycosylation. In particular the component with MW ≈ 80 kD seems to be highly glycosylated possessing poly-*N*-acetyllactosaminglycans. Structural analysis of the polylactosaminoglycans of lamp-positive proteins is currently performed by HPAE-PAD and FAB-MS (supported by the Deutsche Forschungsgemeinschaft Ku 781/2-1 and Eg 39/10).

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S4.13

Epidermal Growth Factor Induces Glycolipid Sulfotransferase Activity in Human Renal Cell Carcinoma Cells

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Accumulation of sulfolipids associated with markedly elevated levels of glycolipid sulfotransferase activities was previously demonstrated in human renal cell carcinoma and a cell line (SMKT-R3) derived therefrom. To elucidate the regulatory mechanism of the sulfolipid synthesis, effect of growth factors on the sulfotransferase activities toward GalCer and LacCer was investigated in SMKT-R3 cells. Exogenous epidermal growth factor (EGF) increased significantly the activity levels of the sulfotransferases in a dose-dependent manner, but did not change that of arylsulfatase A which hydrolyzes the sulfolipids. Metabolic labeling with ^{35}S-sulfate revealed that the addition of EGF resulted in the increment of the sulfolipid synthesis. The expression of the EGF receptor on SMKT-R3 cells was demonstrated by affinity cross-linking with ^{125}I-EGF. From