Sensing dynamic changes in complex tumor cell populations by imaging and flow cytometry: role of NCAM polysialylation-in adherence transitions of small cell lung cancer

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Asymmetric cell division, ongoing cellular variation, microenvironmental influences and stochastic changes in cellular behaviour can mask the origins of tumour progression and therapeutic resistance. Flow and imaging cytometry can provide valuable insights into the complex temporal changes that occur in cell micro-communities either spontaneously or in response to selection pressure. Temporal transitions in cellular adherence are intimately involved in tumour progression and spread - with the consequence that tumour cell populations are intrinsically heterogeneous with respect to transition forms and their linked molecular events. In the case of small cell lung cancer (SCLC) spontaneous transition of anchorage-independent cells to adherent and migratory variant forms has implications for tumour progression and spread.

The reversible decoration of neural cell adhesion molecule (NCAM; NCAM1 or CD56) by polysialic acid (polySia) is thought to permit transitions that enable both neuronal plasticity and cancer cell spread (1). SCLC progression correlates with increased polysialylated NCAM expression. PolySia is a linear alpha-2,8-linked polymer of up to 200 residues of *N*-acetylneuraminic acid (sialic acid, Neu5Ac). Two Golgi-associated polysialyltransferases, ST8SiaII (STX) and ST8SiaIV (PST), separately regulated at the transcriptional level, enable the transfer of sialic acid via an α-2,3-linked sialic acid residue to *N*-linked glycans of the IgV domain of NCAM.

Conceptually, polySia acts as an polyanionic 'anti-adhesive' molecule (2) facilitating detachment and metastatic migration. PolySia-NCAM is an attractive target for modulating tumour cell behaviour (3) since polySia-decoration of NCAM reflects a defined glycosylation pathway and PSA expression is normally non-existent by adulthood. However, the 'anti-adhesive' role of polySia decoration of NCAM during such spontaneous transitions remains unclear. Here we have studied the functional consequences of the loss or gain of polysialylation in different SCLC cell adherent states. The study applies imaging and flow cytometric methods and reveals the different routes for the evolution of heterogeneous polysialylation patterns in dynamic SCLC populations.

We show that variant-generating SCLC cell lines exist in bistable states in which isolation of cells in early stages of adherence transition can resolve populations with either increased or negligible levels of polysialylation. In the classical NCI-H69 cell line, early variant forms showed rapid adherence and loss of decoration. Later acquisition of full adherence was accompanied by re-expression of polysialyation and polySia-cleavage studies showed that adherence was independent of NCAM polysialylation per se, even on preferred extracellular matrix substrates. The early loss of decoration in NCI-H69 reflected a down-regulation of polysialyltransferase gene expression. Acquisition of adherence in NCI-H69 was accompanied by reduced expression of SCLC marker genes and changes in adhesion molecule gene expression as surveyed by microarray analysis. Using a novel nanoparticle dilution approach using Qdot® 705 nanocrystals (4), proliferation kinetics of adherent variants were found to be independent of whether cells were maintained in microclusters or as substrate adherent forms. PolySia-negative NCI-H69 populations, selected for increased growth rate, re-expressed extensive NCAM polysialylation but had the capacity to form tight cell-cell contacts and evolve internal microenvironments with reduced oxygen availability – providing a route to drug resistance. Using a live cell antibody-mimic probe (EndoN-GFP) for assessing polysialylation, micro-community composition was found to modulate the NCAM polysialylation for resident adherent cells, suggesting a route for maintaining bistability.

The dynamic nature of NCAM polysialylation, polySia-independence of adherence and micro-community influences suggest that variant potential is masked in heterogeneous populations but realized upon isolation of cells. We suggest that the realization of variant behaviour occurs during tumour spread. Bistability offers interventions limiting reversal from the adherence transition state. The study recognises for the first time a distinct adherence transition state characterised by reduced polysialyltransferase expression and signature marker gene changes (5).

References

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