

tumour biology and pathology

1655P NEU3 ACTIVITY ENHANCES EGFR ACTIVATION WITHOUT AFFECTING EGFR EXPRESSION

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Aim: The plasma membrane associated sialidase NEU3 is often deregulated in colorectal cancer (CRC) and was demonstrated to coimmunoprecipitate in HeLa cells with the epidermal growth factor receptor (EGFR), the molecular target of the recent therapies based on monoclonal antibodies. Elucidating this correlation may provide novel understandings in cancer biology and may represent a new basis to develop

innovative therapeutic approaches. Therefore, our aim was to evaluate the effect of NEU3 deregulation on EGFR expression and activation in CRC cell lines.

Methods: NEU3 and EGFR expression was evaluated by Real-time PCR (Q-PCR) in 13 human CRC cell lines, as well as in DiFi cells. Regulation of EGFR pathway by NEU3 activity was studied using NEU3 wild-type (wt) and double-mutant (encoding for NEU3 inactivated protein) vectors transiently transfected in DiFi and SW480 cell lines. EGFR gene and mutational status were assessed by FISH and direct sequencing.

Western Blot analysis was performed to study expression and phosphorylation status of EGFR downstream proteins. EGFR sialylation levels were evaluated by lectin binding assay using biotinylated SNA (Sambucus nigra agglutinin) and avidin-horseradish peroxidase. Cell viability was assessed by MTT assay.

Results: We observed up-regulation of NEU3 mRNA levels and EGFR activation in all the investigated cell lines, independent of EGFR mRNA/protein expression or gene status. Transfection assays confirmed that only NEU3 wt enhanced EGFR activation without affecting its expression. Moreover, the level of EGFR sialylation was reduced in cells overexpressing the NEU3 wt sialidase, strongly suggesting that EGFR sialylation is regulated by NEU3. MTT test's results showed a significant increase of cell viability ($p < 0.01$) upon transfection with the wt enzyme, whereas with the double-mutant form, no difference was detected between transfected and control cells.

Conclusions: Since NEU3 reveals to be overexpressed in the totality of cancer cell lines analyzed and its activity regulates the EGFR pathway, we assumed this sialidase as a good and innovative marker for diagnosis and therapy of CRC.

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