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MEETING ABSTRACT

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Deglycosylation of the cytokine co-receptor gp130 reveals a rapid protein turnover, which gives rise to biased signalling

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Background: The signal transducer glycoprotein 130 (gp130) is a common co-receptor for cytokines of the interleukin (IL)-6 family and is heavily *N*-glycosylated. The protein gp130 is expressed ubiquitously, including in cancer cells. Of particular interest, circulating IL-6 levels are elevated in late-stage melanoma patients and predict unfavourable outcome. Interestingly, the cholesterol-lowering drugs, the statins, trigger apoptosis in metastatic melanoma cells, while primary melanoma cells from the radial growth phase are virtually insensitive. Moreover, statins affect the glycosylation machinery in the endoplasmic reticulum by a reduction of endogenous dolichol levels. However, it is unclear whether the glycosylation status of gp130 is altered under these conditions and whether signal transduction is impaired.
Methods: Human melanoma cell lines reflecting early radial growth phase (WM35, WM278, WM793b) and advanced metastatic stages (A375 and 518a2) were investigated for expression of gp130. Glycosylation was probed by PNGase treatment of cell lysates, tunicamycin or simvastatin. Signalling was monitored for phosphorylation of STAT3 and ERK1/2. Treatments were analysed for multiple comparison with ANOVA and *post hoc* Dunnett test for statistical significance at a p value of < 0.05.
Results: The inhibition of *N*-linked glycosylation by tunicamycin resulted in an accelerated migration of gp130 in SDS-PAGE, shifting the 135 kDa band to an apparent molecular mass of 95–100 kDa. In order to confirm deglycosylation, PNGase treatment resulted in a similar pattern of gp130. Deglycosylation by tunicamycin was equally distributed to all melanoma cells, whereas simvastatin-induced deglycosylation was virtually absent in cells from the early disease stage. The kinetics of the deglycosylation revealed a rapid decline of the 135 kDa band with a half-life of 2.5 hours, implicating rapid turnover of this protein. Accordingly, a complete loss of basal STAT3 phosphorylation was observed with tunicamycin, while simvastatin prevented activation only in metastatic melanoma cells. Complementary to these observations, activation of ERK1/2 was significantly augmented in simvastatin-treated metastatic melanoma cells in the presence of IL-6, while primary melanoma cells were hardly affected.
Discussion: In conclusion, simvastatin gives rise to biased signalling via the IL-6 receptor subunit gp130 in a glycosylation-dependent manner.
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