Glycobiology 2020: The heparan sulfate proteoglycan Syndecan-1 and heparanase are novel regulators of cancer stem cell function and therapeutic resistance - Martin Gotte- Munster University Hospital

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Introduction:
The heparan sulfate proteoglycan Syndecan-1 binds cytokines, morphogens and extracellular matrix components, regulating cancer stem cell properties and invasiveness. Syndecan-1 is modulated by the heparan sulfate-degrading enzyme heparanase, but the underlying regulatory mechanisms are only poorly understood (1). In colon cancer pathogenesis, complex changes occur in the expression pattern of Syndecan-1 and heparanase during progression from well-differentiated to undifferentiated tumors. Loss of Syndecan-1 and increased expression of heparanase are associated with a change in phenotypic plasticity and an increase in invasiveness, metastasis and dedifferentiation. Here we investigated the regulatory and functional interplay of Syndecan-1 and heparanase employing siRNA-mediated silencing and plasmid-based overexpression approaches in human colon cancer cell lines and in a xenograft model. Sdc-1 small-interfering RNA knockdown in the human colon cancer cell lines Caco2 and HT-29 resulted in an increased side population (SP), enhanced aldehyde dehydrogenase 1 activity, and higher expression of CD133, LGR5, EPCAM, NANOG, SRY (sex-determining region Y)-box 2, KLF2, and TCF4/TCF7L2. Notably, heparanase expression and activity were upregulated in Syndecan-1 depleted cells. This increase was linked to an upregulation of the transcription factor Egr1, which regulates heparanase at the promoter level. Sdc-1 knockdown enhanced sphere formation, cell viability, Matrigel invasiveness, and epithelial-to-mesenchymal transition-related gene expression. Likewise, upregulation of heparanase increased the colon cancer stem cell phenotype based on sphere formation assays and phenotypic marker analysis (Side-population, NANOG, KLF4, NOTCH, Wnt, and TCF4 expression). Sdc-1 depleted HT-29 xenograft growth was increased compared to controls. Decreased Sdc-1 expression was associated with an increased activation of β1-integrins, focal adhesion kinase (FAK), and wingless-type (Wnt) signaling. Pharmacological FAK and Wnt inhibition blocked the enhanced stem cell phenotype and invasive growth. Sequential flow cytometric SP enrichment substantially enhanced the stem cell phenotype of Sdc-1-depleted cells, which showed increased resistance to doxorubicin chemotherapy and irradiation. In conclusion, Sdc-1 depletion cooperatively enhances expression of heparanase and activation of integrins and FAK, which then generates signals for increased invasiveness and cancer stem cell properties. Our findings provide a new avenue to target a stemness-associated signaling axis as a therapeutic strategy to reduce metastatic spread and cancer recurrence.

Results and Discussion: In colon cancer, downregulation of the transmembrane heparan sulfate proteoglycan syndecan-1 (Sdc-1) is associated with increased invasiveness, metastasis, and dedifferentiation. As Sdc-1 modulates signaling pathways relevant to stem cell function, we tested the hypothesis that it may regulate a tumor-initiating cell phenotype. Sdc-1 small-interfering RNA knockdown in the human colon cancer cell lines Caco2 and HT-29 resulted in an increased side population (SP), enhanced aldehyde dehydrogenase 1 activity, and higher expression of CD133, LGR5, EPCAM, NANOG, SRY (sex-determining region Y)-box 2, KLF2, and TCF4/TCF7L2. Sdc-1 knockdown enhanced sphere formation, cell viability, Matrigel invasiveness, and epithelial-to-mesenchymal transition-related gene expression. Sdc-1-depleted HT-29 xenograft growth was increased compared to controls. Decreased Sdc-1 expression was associated with an increased activation of β1-integrins, focal adhesion kinase (FAK), and wingless-type (Wnt) signaling. Pharmacological FAK and Wnt inhibition blocked the enhanced stem cell phenotype and invasive growth. Sequential flow cytometric SP enrichment substantially enhanced the stem cell phenotype of Sdc-1-depleted cells, which showed increased resistance to doxorubicin chemotherapy and irradiation. In conclusion, Sdc-1 depletion cooperatively enhances activation of integrins and FAK, which then generates signals for increased invasiveness and cancer stem cell properties. Our findings may provide a novel concept to target a stemness-associated signaling axis as a therapeutic strategy to reduce metastatic spread and cancer recurrence. DATABASES: The GEO accession number of the Affymetrix transcriptomic screening is GSE58751.

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