Ruprecht-Karls-Universität Heidelberg Medizinische Fakultät Mannheim Dissertations-Kurzfassung

The role of hyaluronic acid in regulating matrix-assisted and BMP4/7-stimulated Id1 and Id3 expression in melanoma cells

Autor: Ruolin Wu

Institut / Klinik: Zentrum für Biomedizin und Medizintechnik Mannheim (CBTM). Mikrovaskuläre Biologie und Pathobiologie

Doktorvater: Prof. Dr. J. Sleeman

Expression of the bone morphogenetic protein (BMP) target genes Inhibitor of Differentiation (Id)1 and Id3 determines the stemness properties of tumor cells, and is associated with the poor survival of melanoma patients. Previous work in the lab showed that co-injection of melanoma cells into syngeneic mice with artificial matrices such as Matrigel, collagen, and laminin can promote tumor initiation and thus stemness properties. Gene expression profiling of melanoma cells under 3D matrix conditions revealed that the expression of Id1 and Id3 is significantly upregulated via autocrine BMP signalling in these matrices, indicating that stemness properties are strongly dependent on the microenvironment that tumor cells are exposed to. In my MD thesis, I have therefore investigated the role of extracellular matrix in the regulation of BMP signalling in melanoma cells. First, I tested the hypothesis that 3D matrices might act as a physical barrier and prevent the free diffusion of tumor cell-secreted BMP, which leads to BMP accumulation around tumor cells and signalling via BMP receptors in an autocrine manner. Indeed, I found that compared with 2D conditions, 3D Matrigel significantly reduced the diffusion coefficient of fluorescently-labelled BMP2. Second, I tested whether the extracellular matrix component hyaluronan (HA) regulates BMP signalling in melanoma cells, as solid stress in 3D envionments increases HA synthesis by tumor cells, and previous studies with murine chondrocytes have shown that the HA containing pericellular matrix is critical for BMP7 signaling. CD44, the main cell surface receptor for HA, promotes the cellular response to BMP7 stimulation in this context. I therefore studied the role of HA in regulating Id1 and Id3 gene expression. In 3D Matrigel I found that depletion of pericellular HA from the matrix either by exogenous hyaluronidase treatment or ectopic expression of Hyal1 significantly reduced the expression of Id1 and Id3 in melanoma cells.However, inhibition of endogenous HA synthesis by its specific inhibitor 4-MU or blocking the interaction of HA with its receptor CD44 by anti-CD44 antibody KM81 did not result in a reduction of Id1 and Id3 expression in 3D Matrigel culture. However, in 2D culture both exogenous hyaluronidase and 4-MU treatment reduced BMP4 and BMP7-dependent Id1/3 protein expression. Ectopic expression of the Hyal1 gene reduced BMP4 and BMP7-dependent Id1/3 protein expression. Exogenous high molecular weight (HMW)-HA treatment increased BMP4 and BMP7-dependent Id1/3 protein expression, while pathophysiological concentrations of small HA oligosaccharides did not show any inhibitory effects on BMP4 and BMP7-dependent Id1/3 protein expression. Knockdown of CD44 showed a reduction of BMP4 and BMP7-dependent Id1/3 protein expression. Co-immunoprecipitation assays indicated that HMW-HA can promote the physical interaction between CD44 and BMP type II receptor (ActR-II) in the presence of BMP4/7, suggesting that CD44 can act as an HA-dependent co-receptor for BMPs. By analyzing mRNA expression data from skin cutaneous melanoma patients, I found that gene expression of Id1 is significantly correlated with both Id3 and HAS3, and that patients with combined expression of Id3 with either HAS2, HAS3 or CD44 had poorer overall survival than the patients with single expression of these genes. Taken together, these findings strongly suggest that HA and CD44 promote BMP4- and BMP7- dependent Id1/3 protein expression in melanoma cells.