Basal ERK1/2 signaling induces anoikis in colorectal carcinoma cells following constitutive re-expression of the chemokine CXCL12

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Abstract

CXCL12 and CXCR4 signaling plays critical roles in development, homeostasis, and tumor metastasis. We have previously shown that epigenetic silencing of CXCL12 in colorectal and mammary carcinoma promotes metastasis. Anoikis is an essential process of colonic epithelial turnover and limits the metastatic progression of cancer. We sought to determine the role for anoikis in limiting tumor metastasis upon re-expression of CXCL12 in human colorectal carcinoma cell lines. Methods: Lucifase and CXCL12 were expressed in HCT116 and HT29 colorectal carcinoma cells by stable transfection. Tumor formation and metastasis of cecal wall xenografts were monitored using in vivo bioluminescence imaging. CXCL12-induced anoikis was defined using caspase 3/7, focal adhesion kinase (FAK) and p130Cas de-aggregation, DNA fragmentation, and cell survival assays. Phosphorylation of ERK1/2 in native colonic epithelium, non-transformed IEC6 cells, and colorectal carcinoma cells was monitored by immunoblot and immunohistochemistry and was inhibited using the specific MEK inhibitor U0126. Results: Constitutive expression of CXCL12 in human colorectal carcinoma cells reduced orthotopic tumor formation and inhibited metastasis in SCID mice. Further, we show that CXCL12 expression induces anoikis specifically in non-adherent colorectal carcinoma cells. Apoptotic cell death was preceded by hypophosphorylation and de-aggregation of the focal adhesion kinase complex proteins FAK and p130Cas leading to increased cellular de-adhesion in culture. Cellular detachment of carcinoma cells re-expressing autocrine CXCL12 was dependent upon defined alterations in the extracellular matrix. CXCL12-induced anoikis of carcinoma cells was consistent with and dependent upon basal ERK1/2 activation. Those data in colorectal carcinoma cells were subsequently shown to mirror elevated levels of phosphorylated ERK1/2 demonstrated in surface colonic epithelium and normal, non-transformed IEC6 cells. Conclusions: These data provide a mechanism to explain the dichotomy between the physiologic and pathophysiologic roles for CXCL12-CXCR4 signaling in colonic carcinoma cells. In contrast to endocrine CXCR4 signaling in cancer, anoikis of carcinoma cells expressing endogenous CXCL12 was mediated in part upon sustained basal ERK1/2 signaling consistent with physiologic dynamics of the colonic epithelium.

Expression of CXCL12 in human colorectal carcinoma cells

CXCL12 expression increases apoptosis in non-adherent colorectal carcinoma cells

CXCL12 expression modulates extracellular matrix and increases focal adhesion breakdown

CXCL12 expression induces colorectal carcinoma cell anoikis

CXCL12 induces basal ERK1/2 activation which contributes to colorectal carcinoma cell anoikis

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

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