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Role of Chemokines in Colorectal Cancer and its Liver Metastasis

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The rationales for this work are based on previous observations from a rat liver metastasis model, where rat CRC cells were implanted intra-portly into the respective livers and re-isolated after specific time intervals. Following the cDNA microarray analysis of these cells, significant modulations were identified (≥2fold up- or down-regulated) in ~3000 genes, during the first week after implantation. Chemokines, both ligands and receptors, also showed profound alterations in their expressional levels during this study. Out of these significantly altered chemokines, two receptors (CCR5 and CXCR4) and two ligands (CXCL4 and CXCL7) were incorporated and investigated in this work.

Significant siRNA mediated knockdown of the selected genes was accomplished and confirmed at mRNA and/or protein levels. Following the knockdown procedures, different functional assays referring to proliferation, migration, colony formation, cell cycle analysis, apoptosis, nuclear staining etc were performed accordingly. In addition to this, antagonist based blockage of the selected receptors and subsequent effects were also highlighted in this work. CCR5 knockdown or its blockage by the antagonist (maraviroc) induced significant anti-proliferative effects in selected CRC cells. Similarly, this knockdown or blockage of CCR5 also resulted into reduced colony formation and migration of CRC cells in vitro. Blockage of CCR5 also exerted mild cytostatic and significant apoptotic effects in CRC cells. Following the CCR5 blockage, significant up-regulation (≥2fold up) of the cell surface death receptors (FAS, TNF),

mitochondrial stress related genes (BCL2 family) and activated caspases (3, 7 and 9) were observed. Following the implantation of CRC cells (CC531) into rat livers and subsequent treatment with maraviroc, remarkably, the growth of these cancer cells was eliminated in the respective rats. CXCR4 knockdown and its blockage by antagonist (AMD3100) showed anti-proliferative effects. Knockdown or blockage of CXCR4 also inhibited the colony formation mildly, while migration was inhibited significantly. Migratory behavior of the CRC cells was also influenced by a newly identified CXCR4 ligand (ubiquitin), where CRC cells showed a high migration rate towards the ligand source. Significant signs of apoptosis were also witnessed after the CXCR4 blockage in CRC cells. Knockdown of CXCL4 and CXCL7 inhibited the proliferation of CRC cells, but the effects were more profound in human CRC cells for both genes. Inhibition of colony formation was also observed in CRC cells after the knockdown of CXCL4 or CXCL7. Similarly, knockdown of the two chemokine ligands reduced the migration of CRC cells towards a source of growth factors (FBS). Moreover, inhibition of CXCL4 and CXCL7 induced apoptotic signs in CRC cells including the detachment from culture plates, condensation of nuclei and fragmentation of DNA.

In summary, these results highlight the importance of chemokines, their role in CRC and its liver metastasis. Blocking the chemokine receptors, like CCR5, by antagonists can be a promising field in cancer treatment. In this regard, the clinically approved maraviroc is to be considered as an immediately available CCR5 antagonist for treatment of CRC metastasis in patients. Targeting the ubiquitin-CXCR4 axis in CRC is also important in a way that both of these components are produced by a variety of leukocytes and cancer cells. Focus on platelets factors like CXCL4 and CXCL7, is also an interesting aspect in CRC, as the two markers are often associated with CRC especially in its liver metastasis.