Targeting integrins with anti-metastatic drug candidates and control of colon cancer dissemination.

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Therapeutic strategies aimed at targeting specific determinants of metastatic cells are today the main approach to tumour treatment[1,2]. In this study we examine the role of integrins in the dissemination of colon cancer metastases into the liver, and the use of metal-based compounds to identify new therapeutic targets to control this process.
We investigated the interactions of tumour cells with the extracellular matrix (ECM) components *in vitro*, using the human metastatic HCT116 colon cancer cell line, and the healthy hepatic cell line IHH. HCT-116 cells significantly adhere to the main components of the hepatic ECM, such as fibronectin and collagen I. Since integrins are the main adhesion receptors for the cell-matrix interactions[3], and specifically α5β1 integrin is the main receptor for fibronectin, we evaluated its role in a model simulating colon cancer cell dissemination. The adhesion of HCT-116 cells towards fibronectin significantly increases by more than 100% ($p<0.005$), when cells are grown in the medium conditioned by healthy hepatocytes. To analyze this event, we investigated the activation of FAK, the intracellular kinase directly correlated to the activation of α5β1 integrin[4]. Data obtained confirmed the hypothesis that soluble factors released by hepatocytes, synergize with the presence of fibronectin, inducing the increase of FAK phosphorylation in HCT-116 cells by 37% as compared to the same medium un-conditioned by hepatocytes. Moreover, soluble factors released by hepatocytes induce α5β1 to influence the activity of other integrins involved in the HCT-116 cell-ECM interactions. In fact, we observed the reduction of cell adhesion also to collagen I when HCT-116 cells were grown in the medium conditioned by healthy hepatocytes, an event that points out on possible cross-talk interactions between α5β1 and α2β1 integrins.
To investigate if alfa5beta1 integrin could be proposed as a target for new anti-metastatic drugs, we took advantage from the features of NAMI-A, a ruthenium-based compound selectively active towards secondary cancers in a number of experimental models of solid tumours *in vivo* and in humans[5]. Morphological analyses performed by atomic force microscopy on HCT-116 tumour cells treated with NAMI-A, indicate a significant increase in tumour cell volume and a more rounded shape, in comparison to untreated controls. These data are in line with previous results obtained using other tumour cell lines, in which these morphological changes correlated with cytoskeletal rearrangements and the modulation of integrin activity. NAMI-A is able to modulate also the integrin profile of HCT116 and, in addition, it reduces by 50% ($p<0.05$) and by 35% tumour cell adhesion to fibronectin, respectively at 1 uM and 10 uM concentrations. Correspondingly, at the same concentrations NAMI-A decreases alfa5beta1 integrin levels on cell surface, and reduces the phosphorylation of FAK. To further investigate if NAMI-A alters the expression level of the gene *FAK* and of the single subunits of the integrin alfa5beta1, Real time PCR analyses are in progress.
Results since now obtained confirm the important role of integrins, and particularly of alfa5beta1 integrin, in colon cancer dissemination and suggest that the anti-metastatic activity of NAMI-A can depend on the alteration of integrin functions of these tumour cells. These data suggest integrins as the target molecules for the development of new therapeutic strategies.

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References

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