Abstract

The ability of cancer cells to adopt various invasive modes (the plasticity of cancer cell invasiveness) represents a significant obstacle in the treatment of cancer metastasis. Cancer invasiveness involves various modes of migration. Cells can move together (with the preserved intercellular junctions; collective invasiveness) or individually. Within individual invasiveness, we distinguish two principal invasive modes – mesenchymal and amoeboid. The mesenchymal mode of migration is characterized by an elongated shape, proteolytic degradation of the fibres of the extracellular matrix, and the formation of strong contacts with the extracellular matrix. The amoeboid mode of migration is not dependent on proteolytic activity, the cells are characterized by a round shape and increased contractility, which they use to squeeze themselves through the pores of the extracellular matrix. This thesis deals with the analysis of the plasticity of cancer cell invasiveness, specifically the transitions between individual amoeboid and mesenchymal migration modes, in the 3D environment of the collagen gel as a model of extracellular matrix. The work presents models of mesenchymal-to-amoeboid transition (MAT), which include BLM, HT1080 and MDA-MB-231 cell lines, in which MAT is induced by the expression of constitutively active small GTPase RhoA or by the treatment with the Src inhibitor dasatinib. A non-cancer model of cell plasticity based on M2 macrophages in collagen of different density was also established. Subsequently, transcriptomic and proteomic analysis of selected models was performed. These analyses revealed increased expression of proinflammatory genes and decreased expression of cell cycle regulating genes in amoeboid cells. The gene expression profile and the level of protein products of selected targets involved in MAT were subsequently verified using RT-qPCR and immunoblotting. Subsequent comparison of the very diverse publicly available transcriptomic datasets analysing the transition between mesenchymal and amoeboid mode of invasion in a 3D environment revealed increased expression of the long non-coding RNA MALAT1 (Metastasis-Associated Long Adenocarcinoma Transcript 1) in amoeboid cells. This observation was subsequently verified by RT-qPCR. To further investigate the role of MALAT1 in the invasive plasticity of cancer cells, clones with decreased level of MALAT1 expression derived from strongly amoeboid cell lines A375m2 and A2058 were prepared. In both sets of clones, a decrease in MALAT1 expression led to a decrease in the amount of active RhoA (the high level of RhoA is a typical characteristic of amoeboid invasiveness), and also to an increase in the proliferation rate of these cells. In addition, in clones derived from the A375m2 line, a decrease in MALAT1 levels led to an amoeboid-to-mesenchymal transition (AMT) and an increase in the invasiveness of these cells in the 3D environment of the collagen matrix.