Abstract of the doctoral dissertation

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‘Induction of differentiation of monocytes into macrophages by colon cancer cells’ secreted factors’

Tumor in the organism develops in the microenvironment which is composed by cancer cells as well as normal cells. They make specific niche that triggers two important processes: an escape of tumor cells from immune surveillance and the formation of blood and lymphatic vessels.

During the development of the tumor disease, monocytes and macrophages circulating in the bloodstream or occurring in a tissue are recruited actively into the tumor microenvironment. There, due to various microenvironment signals from tumor cells and stroma, the macrophage phenotype can differentiate from anticancer, pro-inflammatory subset (M1-like) into immunosuppressive form that promotes the development and progression of cancer (M2-like).

The term ‘tumor-associated macrophages’ (TAMs) is suitable for macrophages characteristic for the tumor microenvironment. It is a heterogeneous population of cells that acts differentially on the molecular and functional level, depending on the local microenvironment. In the initial stage of cancer development TAMs have the M1 phenotype and have an anti-cancer effects. The colon cancer soluble factors cause the differentiation of TAMs into M2-like, immunosuppressive subset. TAMs have characteristic surface molecules and take a significant part in the production of metalloproteinases of extracellular matrix, cytokines, chemokines and heat shock proteins.

In numerous studies it is suggested that accumulation of tumor associated macrophages correlates with a poor prognosis, although there are contradictory information about their role in colon cancer development, so the further studies and analysis are needed to be conducted.

Therefore, the aim of this study was to evaluate how the soluble colon cancer derived factors influence the monocytes differentiation to macrophages by specifying if cancer promotes the differentiation to pro-inflammatory, anticancer M1 subset, or immunosuppressive and pro-tumorigenic M2 subset. The other aim was to determine if the stage of colon cancer influences the differentiation.

In the research, the following cell lines were used: HT29, LS180, SW948 and SW620. They represented the four following stages of colorectal adenocarcinoma. HSF line, derived from human skin fibroblasts, was used as a control. The culture media (CM, conditioned media) from the lines mentioned above became the model of the tumor microenvironment. An acute monocytic leukemia cell line, THP-1, was examined as a model of human monocytes, and PMA-preactivated monocytes were a model of resting macrophages.

In the first stage of the study, the effect of the conditioned media (CM) on viability (iodide propidium staining, PI), production of free radicals (DCF staining) and expression of surface markers on monocytes and macrophages was investigated by flow cytometry method depending on incubation period. Based on the obtained results 72 hours was chosen for further studies. Secondly, antiproliferative properties of CM from colon cancer tumor cells on monocytes and macrophages were also examined by the succinate dehydrogenase activity (MTT) and the thymidine analog incorporation tests (BrdU).

Based on the results of the cell proliferation assays, CM ability to modulate the cell cycle progression was examined by flow cytometry using propidium iodide staining (PI/RNAse). Furthermore, changes in cell morphology under tumor CM exposure were determined using May-Grünwald- Giemsa staining. In the further studies, the influence of CM dose on phenotype of monocytes and macrophages was investigated. By flow cytometry the influence on viability, production of free radicals and expression of characteristic surface and intracellular molecules (CD11b, CD163, CD206, CD14, CD204, B7H1, CD40, HLA-DR, IDO, CD68) was examined.

The next test which helped to estimate the level of monocytes and macrophages-produced cytokines (VEGF, IL-10 and IL-6) was the ELISA assay. By the qRT-PCR method, the influence of CM on the expression of genes encoding protein markers of the tumor development and metastasis (*iNOS, ARG1, IDO1, LGALS3* and *LGALS9*) in monocytes and macrophages was determined.

In the last stage of the study, the direct influence of CM on production of proteins associated with metastasis (galectin-3 and galectin-9) and metalloproteinases (MMP-9) was tested by, respectively, Western blotting and gelatin zymography.

The following results of the experiments suggest that colon cancer conditioned media induce monocytes differentiation to macrophages, decrease proliferation of monocytes and cause the cell cycle arrest in the G_1 stage. Also CM induce increased production of reactive oxygen species and expression of molecules characteristic for M1 and M2 cell subsets. The

studies showed the increased gene expression and production of the cytokines, galectins and metalloproteinases (cancer progression involved proteins) by macrophages.

In most experiments results, all lines showed strong influence on monocytes and macrophages, especially the strongest impact of the HT29 line on proliferation and markers expression and the metastatic SW620 line on galectin expression.

The general conclusion from the above results is that the tumor microenvironment can be composed by a mixture of monocyte and macrophage cells with different phenotypes: M1, M2 and TAMs.

Taking into account the obtained results of these studies in the *in vitro* model, one can indicate potential ways of developing new drugs and strategies of the therapy of colorectal cancer, consisting in the inhibiting or reversing of transformation of monocytes and pro-inflammatory macrophages into anti-inflammatory cells, promoting cancer development.