

Changes in the gene expression of co-cultured human fibroblast cells and osteosarcoma cells: the role of microenvironment

Viviana Salvatore¹, Stefano Focaroli¹, Giulia Adalgisa Mariani¹, Maria Carla Mazzotti², Mirella Falconi¹

¹Dipartimento Scienze Biomediche e Neuromotorie, Università, Bologna, Italy - ²Dipartimento di Scienze Mediche Chirurgiche, Università, Bologna, Italy

The progression of malignant tumors does not depend exclusively on the autonomous properties of cancer cells; it is also influenced by tumor stroma reactivity and is under strict microenvironmental control. By themselves, stromal cells are not malignant, and they maintain normal tissue structure and function. However, through intercellular interactions or by paracrine secretions from cancer cells, normal stromal cells acquire abnormal phenotypes that sustain cancer cell growth and tumor progression. In their dysfunctional state, fibroblast and immune cells produce chemokines and growth factors that stimulate cancer cell growth and invasion. In our previous work (1), we established an *in vitro* model based on a monolayer co-culture system of healthy human fibroblasts (HFs) and human osteosarcoma cells (the MG-63 cell line) that simulates the microenvironment of tumor cells and healthy cells. The coexistence between MG-63 cells and HFs allowed us to identify the YKL-40 protein as the main marker for verifying the influence of tumor cells grown in contact with healthy cells.

In this study, we evaluated the interactions of HFs and MG-63 cells in a transwell co-culture system over 24 h, 48 h, 72 h, and 96 h. We analyzed the contributions of these populations to the tumor microenvironment during cancer progression, as measured by multiple markers. We examined the effect of siRNA knockdown of YKL-40 by tracking the subsequent changes in gene expression within the co-culture. We validated the expression of several genes, focusing on those involved in cancer cell invasion, inflammatory responses, and angiogenesis: TNF alpha, IL-6, MMP-1, MMP-9, and VEGF. We compared the results to those from a transwell co-culture without the YKL-40 knockdown.

In a pro-inflammatory environment promoted by TNF alpha and IL-6, siRNA knockdown of YKL-40 caused a down-regulation of VEGF and MMP-1 expression in HFs.

These results suggest that the tumor microenvironment has an influence on the protein expression of healthy surrounding tissues and on the process of tumorigenicity. The mechanisms of the microenvironment are emerging as attractive targets for therapeutic strategies.

References

- [1] Simulating tumor microenvironment: changes in protein expression in an *in vitro* co-culture system. Salvatore V, Teti G, Bolzani S, Focaroli S, Durante S, Mazzotti MC, Falconi M. *Cancer Cell Int.* 2014 May 13;14:40. doi: 10.1186/1475-2867-14-40. eCollection 2014.

Keywords

Tumor microenvironment; YKL-40; inflammation; siRNA; osteosarcoma cells; human fibroblasts.