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Epithelial and mesenchymal cancer cells block adipose stem cells differentiation

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The tumour microenvironment consists of different factors that can contribute to neoplastic transformation, tumour growth and invasion by soluble factors, signalling molecules, and extracellular matrix. Adipose tissue is a rich source of multipotent mesenchymal stem cells termed adipose stem cells (ASCs). Studies showed that MSCs can promote tumour progression and metastasis, while others reported that MSCs suppress tumour growth. Therefore, molecular mechanisms that link MSCs to tumour cells growth are poorly understood.

The aim of this study is to establish the effect of tumour microenvironment on normal human mesenchymal stem cells using co-cultures of osteosarcoma and breast cancer cells with ASCs.

SAOS2, osteosarcoma cell line and MCF7, breast cancer cell line, were co-cultured with ASCs using an insert of 0,4μm at same cell density for 7, 14 and 21 days. Growth curves, stemness gene expression including OCT4, Sox2, and Nanog, leptin expression by RT-PCR, mesenchymal markers expression including CD34, CD29, CD90 and vimentin by flow cytometry, CD324, Twist and Snail, EMT-related markers, by RT-PCR were analyzed. In addition, angiogenic and adipogenic differentiation assays were performed after 21 days of co-culture. In addition, SMAD and p-SMAD2/3 were assayed by western blotting. Angiogenic gene expression was also analyzed by RT-PCR. The results showed that both SAOS2 and MCF7 cells induced an increase of ASCs proliferation respect to ASCs cultured alone. CD90, CD29 and vimentin expressions were similar in all tested conditions, with a mean percentage of 98%. CD324, an epithelial marker, was not expressed as well as no change of Twist and Snail was detectable. Interestingly, SAOS2 and MCF7 cells induced a variation of CD34 expression both at molecular and proteic levels. In particular, at 7 days, MCF7 induced an increase of CD34 gene expression, at 14 days a decrease and at 21 days a strong de novo increase with respect to ASCs cultured alone: the latter was also confirmed by flow cytometry. On the contrary, at 7 days, SAOS2 induced a decrease of CD34 gene expression, but at 14 and 21 days, induced a strong increase by RT-PCR. At proteic level, SAOS2 induced an increase of CD34 in culture time. For stemness genes, both MCF7 and SAOS2 induced an increase of OCT4, Nanog and Sox2 with respect to ASCs cultured alone and a decrease of angiogenic factors, including CD31, PDGFα, PDGFRα, PDGFRβ with respect to ASCs cultured alone, with a significant increase of VEGF at 21 days ($p<0.001$). Leptin was increased in all conditions, whereas both SMAD and pSMAD2/3 increased only on ASCs were cultured alone. After 21 days of coculture, only ASCs cocultured were able to differentiate both in adipocytes and endothelial cells.

Taken together, our results indicate that both mesenchymal and epithelial cancer cells are able to maintain the stemness of ASCs upregulating CD34 expression and inhibiting the angiogenic differentiation.

References

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Keywords

Cancer cells; adipose stem cells; differentiation; angiogenesis; SMAD pathway; microenvironment.