

Short Communication

Comparative Analysis of IL-8 and CXCL-1 Production by Normal and Cancer Stromal Fibroblasts

(chemokine / cancer-associated fibroblasts / cancer microenvironment / wound healing / cancer stroma)

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Abstract. It has been shown that fibroblasts within the stroma of malignant tumours can affect the tumour's biological character, influencing such properties as local aggressiveness and metastasis potential. This influence is asserted via paracrine secretion of multiple cell factors, including chemokines. This study demonstrates that both normal keratinocytes and cancer cells can stimulate the secretion of chemokines IL-8 and CXCL-1 from normal dermal fibroblasts and stromal fibroblasts from squamous cell carcinoma. The effect of epithelia on normal fibroblasts leads to a transient secretory change, in contrast to stromal fibroblasts which generate a more prolonged one. This observation demonstrates that stimulated expression of both IL-8 and CXCL-1 is not specific to cancer, supporting the hypothesis that similar mechanisms exist between wound healing and oncogenesis. It also shows that stromal fibroblasts isolated from a tumour have significantly different features from normal fibroblasts.

Introduction

Malignant tumours represent an incredibly complex system (Egeblad et al., 2010). Like normal tissue, they contain a pool of stem cells (Sell, 2010) and a supportive structure within the tumour stroma. This stromal microenvironment can influence the biological properties of a tumour, such as its local invasiveness and metastasis potential (Plzák et al., 2010). The tumour stroma itself consists of fibroblasts secreting extracellular matrix and chemokines, infiltrating inflammatory cells, and vessels supplying the tumour with oxygen and nutrients (Polyak et al., 2009; Plzák et al., 2010). Activation of the stroma in SCC has been shown to correlate with the activation of genes in cancer cells associated with poor prognosis (Valach et al., 2012). In our previous research, we demonstrated that fibroblasts isolated from squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) have significantly different transcriptomes from normal fibroblasts, and are biologically active toward epithelial cells (Lacina et al., 2007a,b; Strnad et al., 2010; Szabo et al., 2011).

Chemokines produced by both cancer and stromal cells are able to stimulate the pro-inflammatory milieu, thus supporting tumour progression (Mishra et al., 2011). Both normal keratinocytes and FaDu cells derived from SCC stimulate expression of IL-8 and CXCL-1 (GRO-1 oncogene). These secretions are also significantly up-regulated in SCC samples obtained from patients (Kolář et al., 2012). In this paper, we demonstrate how normal fibroblasts and those prepared from the stroma of SCC secrete chemokines IL-8 and CXCL-1 when cultured alone or co-cultured with either normal or malignant keratinocytes.

Material and Methods

Cell Culture

We used the normal dermal fibroblasts (DF), fibroblasts isolated from SCC (SCCF) and normal human keratinocytes (HK) described in our previous studies

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Abbreviations: BCC – basal cell carcinoma, CXCL-1 – chemokine (C-X-C motif) ligand 1, DF – dermal fibroblasts, DMEM – Dulbecco's modified Eagle's medium, ELISA – enzyme-linked immunosorbent assay, FBS – foetal bovine serum, HK – normal human keratinocytes, IL-8 – interleukin 8, SCC – squamous cell carcinoma, SCCF – fibroblasts isolated from squamous cell carcinoma, TRITC – tetramethylrhodamine isothiocyanate.

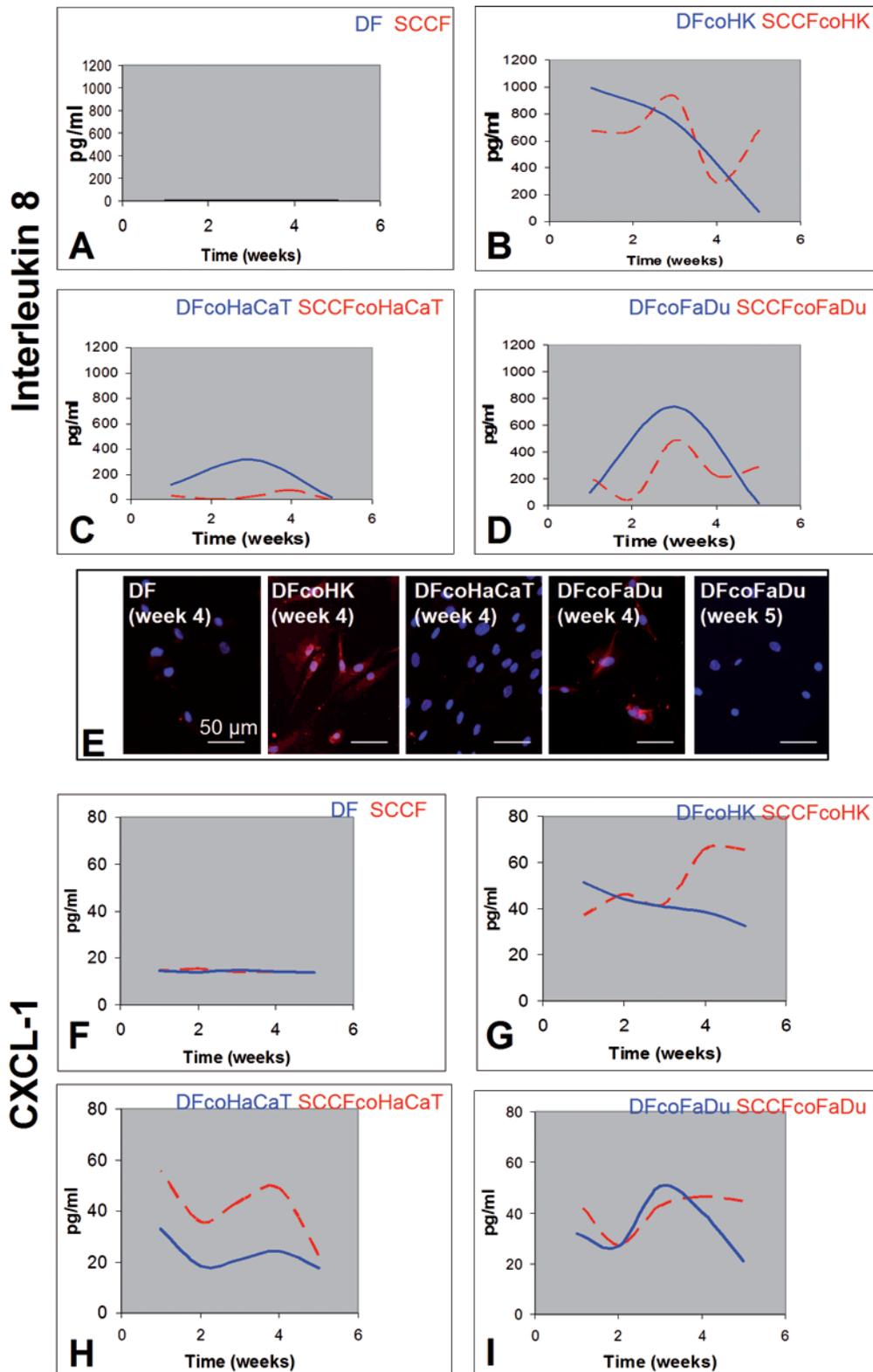


Fig. 1. Secretion of IL-8 (A-D) including immunocytochemical verification (E) and of CXCL-1 (F-I) by dermal fibroblasts (DF, blue line) and fibroblasts isolated from squamous cell carcinoma (SCCF, dashed red line) over the course of 5-week culture. The pure fibroblasts (DF, SCCF) and fibroblasts co-cultured with normal human keratinocytes (DFcoHK, SCCFcoHK), immortal non-tumorigenic keratinocytes (DFcoHaCaT, SCCFcoHaCaT) and squamous cell carcinoma cells (DFcoFaDu, SCCFcoFaDu) were evaluated. Cell nuclei were counterstained with DAPI (bar is 50 μ m).

(Strnad et al., 2010; Kolář et al., 2012). The cells were collected after informed consent of donors according to the Helsinki Declaration. The commercially available

FaDu cell line of a hypopharyngeal carcinoma was obtained from American Type Culture Collection (No. HTB-43; Manassas, VA), and the immortalized but non-

malignant aneuploid keratinocyte (HaCaT) line (Boukamp et al., 1988) was obtained from Deutsche Krebsforschungszentrum (CLS No. 300493; Heidelberg, Germany).

DF and SCCF were cultured alone at 1,000 cells/cm² density or in co-culture with HaCaT (10,000 cells/cm²), FaDu (2,000 cells/cm²) or HK (40,000 cells/cm²) using a trans-well system (Corning, Tewksbury, MA) in Dulbecco's modified Eagle's medium (DMEM, Biochrom, Berlin, Germany) with 10% foetal bovine serum (FBS, Biochrom, Berlin, Germany) at 37 °C and 5% CO₂ for five weeks. More details are described in the paper by Kolář et al. (2012). The density of epithelial cells was determined on the basis of growth kinetics for the distinct epithelial cell types. The passage of both fibroblast types was performed once per week. Before the subculture, samples of metabolized media (48 h) were collected and stored at -20 °C for further analysis of IL-8 and CXCL-1. DF cultured as mentioned above were also used for immunocytochemical analysis.

Immunocytochemistry

DF and SCCF were briefly fixed with paraformaldehyde. Goat anti-donkey polyclonal antibody against IL-8 diluted as recommended by the supplier (R&D Systems, Minneapolis, MN) and donkey anti-goat antibody labelled with TRITC (Jackson Laboratories, West Grove, PA) were used. The specificity of reaction was tested by omission of the first step antibody. The cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, Prague, Czech Republic) and specimens were mounted into Vectashield (Vector Laboratories, Burlingame, CA). The Eclipse 90i fluorescence microscope (Nikon, Prague, Czech Republic) equipped with filterblocks for TRITC and DAPI, and the Cool-1300Q CCD camera (Vosskühler, Osnabrück, Germany) were used for specimen inspection. Data was analysed using LUCIA 5.1 software (Laboratory Imaging, Prague, Czech Republic).

Detection of IL-8 and CXCL-1 in culture media

IL-8 and CXCL-1 were measured by ELISA (R&D Systems, Minneapolis, MN) according to the procedure provided by the producer. The technical duplicates were measured.

Results and Discussion

In pure DF and SCCF cultures, IL-8 production was negligible and CXCL-1 production was very low (Fig. 1 A, F). The introduction of epithelial cells to the cultivation had a distinct stimulatory effect on the production of both chemokines (Fig. 1 B-D, G-I). In the DF co-culture, the chemokine up-regulation had only a transient character when compared to the SCCF co-culture. For SCCF, especially when combined with the HK or FaDu cell lines, the chronologic progression of both chemokines was sustained (Fig. 1 B-D, G-I). The IL-8 expression results for HF at the cellular level were con-

firmed by immunocytochemistry (Fig. 1 E). The HaCaT cell line influenced both fibroblast types similarly. These observations are in agreement with our prior published data which demonstrated the short-term stimulatory effect of normal and malignant epithelia on both types of fibroblasts (Kolář et al., 2012). This data can be used to compare certain similarities between wound healing and oncogenesis, a discussion that has been underway for quite a long time (Dvorak, 1986).

It should be mentioned here that the down-regulation of chemokine concentration in the DF co-culture after four weeks of cultivation can be interpreted as temporal programming of DF that is in contrast with prolonged production of both studied factors IL-8 and CXCL-1 by SCCF. Although the explanation that temporary stimulation of HF reflects the programming for wound healing is rather speculative, it offers some interpretation. These discrete differences between SCCF and DF further complement previous observations where SCCF bioactivity persisted after repeated passaging without making contact with cancer epithelium (Lacina et al., 2007a,b; Strnad et al., 2010). This different reactivity of SCCF toward epithelial cells can be partially explained by hypothetical different origins of cancer-associated fibroblasts, to include macrophages, mesenchymal stem cells, pericytes, endothelial cells, and tumour epithelia (Haviv et al., 2009). Another explanation could be based on epigenetic modification of these cells when compared to normal DF (Hu et al., 2005).

In conclusion, SCCF differ from normal fibroblasts in the production of chemokines important in forming the bioactive microenvironment of the tumour stroma. The interruption of the crosstalk between these fibroblasts and cancer cells could be a potential target for future anticancer therapeutic efforts (Plzák et al., 2010).

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References

- Boukamp, P., Petrussevska, R. T., Breitkreuz, D., Hornung, J., Markham, A., Fusenig, N. E. (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol.* **106**, 761-771.
- Dvorak, H. F. (1986) Tumors: wounds that do not heal. Similarities between tumour stroma generation and wound healing. *New Engl. J. Med.* **315**, 1650-1659.
- Egeblad, M., Nakasone, E. S., Werb, Z. (2010) Tumours as organs: complex tissues that interface with the entire organism. *Dev. Cell* **18**, 884-901.
- Haviv, I., Polyak, K., Qiu, W., Hu, M., Campbell, I. (2009) Origin of cancer associated fibroblasts. *Cell Cycle* **8**, 589-595.
- Hu, M., Yao, J., Cai, L., Bachman, K. E., van den Brùle, F., Velculescu, V., Polyak, K. (2005) Distinct epigenetic changes in the stromal cells of breast cancers. *Nat. Genet.* **37**, 899-905.

- Kolář, M., Szabo, P., Dvořánková, B., Lacina, L., Gabius, H.-J., Strnad, H., Šáňková, J., Vlček, Č., Plzák, J., Chovanec, M., Čada, Z., Betka, J., Fík, Z., Pačes, J., Kovářová, H., Motlík, J., Jarkovská, K., Smetana, K. Jr. (2012) Upregulation of IL-6, IL-8 and CXCL-1 production in dermal fibroblasts by normal/malignant epithelial cells *in vitro*, immunohistochemical and transcriptomic analyses. *Biol. Cell* **104**, 738-751.
- Lacina, L., Smetana, K. Jr., Dvořánková, B., Pytlík, R., Kideryová, L., Kučerová, L., Plzák, Z., Štork, J., Gabius, H.-J., André, S. (2007a) Stromal fibroblasts from basal cell carcinoma affect phenotype of normal keratinocytes. *Br. J. Dermatol.* **156**, 819-829.
- Lacina, L., Dvořánková, B., Smetana, K. Jr., Chovanec, M., Plzák, J., Tachezy, R., Kideryová, L., Kučerová, L., Čada, Z., Bouček, J., Kodet, R., André, S., Gabius, H.-J. (2007b). Marker profiling of normal keratinocytes identifies the stroma from squamous cell carcinoma of the oral cavity as a modulatory microenvironment in co-culture. *Int. J. Rad. Biol.* **83**, 837-848.
- Mishra, P., Banerjee, D., Ben-Baruch, A. (2011) Chemokines at the crossroads of tumor-fibroblast interactions that promote malignancy. *J. Leukoc. Biol.* **89**, 31-39.
- Polyak, K., Haviv, I., Campbell, I. G. (2009) Co-evolution of tumor cells and their microenvironment. *Trends Genet.* **25**, 30-38.
- Plzák, J., Lacina, L., Smetana, K. Jr., Chovanec, M., Dvořánková, B., Szabo, P., Čada, Z. (2010) Epithelial-stromal interaction in squamous cell epithelium-derived tumours: an important new player in the control of tumor biological properties. *Anticancer Res.* **30**, 455-462.
- Sell, S. (2010) On the stem cell origin of cancer. *Am. J. Pathol.* **176**, 2584-2594.
- Strnad, H., Lacina, L., Kolář, M., Čada, Z., Vlček, Č., Dvořánková, B., Betka, J., Plzák, J., Chovanec, M., Šáňková, J., Valach, J., Urbanová, M., Smetana, K. Jr. (2010) Head and neck squamous cancer fibroblasts produce growth factors influencing phenotype of normal human keratinocytes. *Histochem. Cell Biol.* **133**, 201-211.
- Szabo, P., Kolář, M., Dvořánková, B., Lacina, L., Štork, J., Vlček, Č., Strnad, H., Tvrdek, M., Smetana, K. Jr. (2011) Mouse 3T3 fibroblasts under the influence of fibroblasts isolated from stroma of human basal cell carcinoma acquire properties of multipotent stem cells. *Biol. Cell* **103**, 233-248.
- Valach, J., Fík, Z., Strnad, H., Chovanec, M., Plzák, J., Čada, Z., Szabo, P., Šáňková, J., Hroudová, M., Urbanová, M., Štefl, M., Pačes, J., Mazánek, J., Vlček, Č., Betka, J., Kaltner, H., André, S., Gabius, H.-J., Kodet, R., Smetana, K. Jr., Gál, P., Kolář, M. (2012) Smooth muscle actin-expressing stromal fibroblasts in head and neck squamous cell carcinoma: increased expression of galectin-1 and induction of poor-prognosis factors. *Int. J. Cancer* **131**, 2499-2508.