

Review

Epithelial–Stromal Interaction in Squamous Cell Epithelium-derived Tumors: An Important New Player in the Control of Tumor Biological Properties

JAN PLZÁK^{1,2,3}, LUKÁŠ LACINA^{2,3,4}, MARTIN CHOVANEC^{1,2,3}, BARBORA DVOŘÁNKOVÁ^{2,3}, PAVOL SZABO², ZDENĚK ČADA^{1,2} and KAREL SMETANA Jr^{2,3}

¹Department of Otorhinolaryngology, Head and Neck Surgery and ²Institute of Anatomy and ⁴Department of Dermatovenereology, First Faculty of Medicine, and ³Center of Cell Therapy and Tissue Repair, Second Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Abstract. *Mesenchymal–epithelial interaction is important in the morphogenesis of squamous epithelia and their appendages, and in the control of the hair cycle postnatally. This review summarizes data regarding the interaction between stromal fibroblasts and tumor cells, with an emphasis on tumors originating from squamous epithelium. Tumor stromal fibroblasts as important element of the cancer stem cell niche are able to participate in the control of the biological properties of tumors. We propose these stromal cells and their products as novel targets for anticancer therapy.*

Nonmelanoma skin cancer (predominantly basal and squamous cell carcinoma) represents the most frequent malignant tumor worldwide. It is generally accepted that its incidence is increasing with the ageing of the population, but also with climatic and behavioral changes. Although they are less aggressive than tumors originating from the mucosa, their locally aggressive growth frequently mutilates the patient (Figure 1). The carcinomas originating from mucosal regions of the head and neck (predominantly of squamous type) are characterized by lymphatic and distant metastasis in addition to locally aggressive invasion, resulting in a poor prognosis.

Correspondence to: Karel Smetana Jr., Institute of Anatomy, First Faculty of Medicine, Charles University in Prague, U nemocnice 3, 128 00 Prague 2, Czech Republic. e-mail: karel.smetana@lf1.cuni.cz / Jan Plzák, Department of Otorhinolaryngology, Head and Neck Surgery, First Faculty of Medicine, Charles University in Prague, V Úvalu 84, 150 00 Prague 5, Czech Republic. e-mail: jan.plzak@lf1.cuni.cz

Key Words: Cancer-associated fibroblasts, cancer microenvironment, tumor stroma, squamous cell carcinoma, basal cell carcinoma, review.

Despite the progress in both diagnosis and treatment, no significant progress has been achieved, except when the treatment is started at an advanced stage of the disease. New results from investigation of different types of tumors indicate the importance of tumor microenvironment on the biological properties of tumor, such as the local aggressive growth and lymphatic or distant metastasation. This microenvironment is highly complex and includes many cell types such as fibroblasts, inflammatory and endothelial cells and their products such as cytokines and extracellular matrix. This review article summarizes data about the role of the epithelial–mesenchymal interaction in tumor originating from squamous epithelium.

Epithelium–Matrix Interaction under Physiological Conditions

Squamous epithelium covers the mechanically stressed areas of external (skin) or internal (oral cavity, vocal cords, oesophagus *etc.*) surfaces of the human body. This epithelium is morphologically and functionally stratified, reflecting the functional properties of this tissue. The proliferating cells are located in the basal layer of epithelium under normal physiological conditions. These proliferating cells also include a pool of epidermal stem cells (SCs). In addition to the basal layer of glabrous areas, epidermal SCs are also present in hairy skin in the bulge region of the outer root sheath of the hair follicle (1, 2). Epidermal SCs are slowly cycling cells, and their division has an asymmetrical character. The arising daughter cells have either the character of epidermal SCs (responsible for self-renewal and long-lasting maintenance of the tissue) or so-called transit-amplifying cells (TACs), which start to differentiate. TACs proliferate quickly, but the number of mitotic divisions is restricted (3, 4).

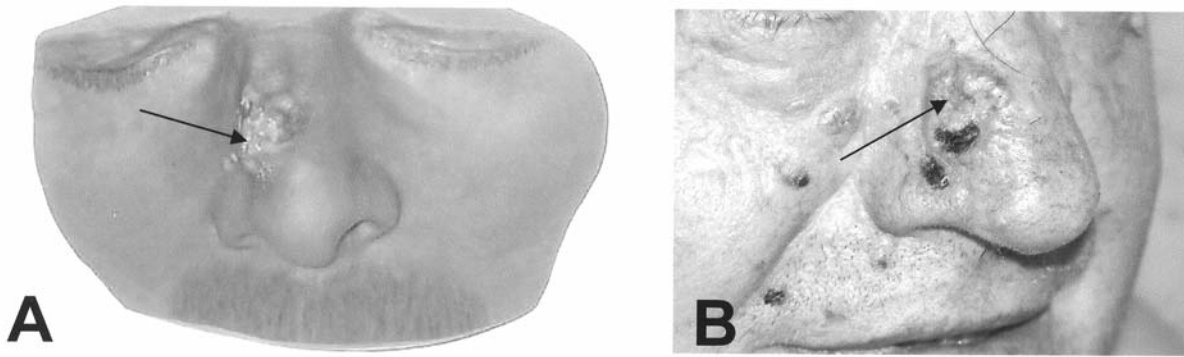


Figure 1. Comparison of the appearance of advanced basal cell carcinoma of the nose 100 years ago (A) and today (B). The tumor is marked by an arrow. 100-year-old model (A) was prepared from a specimen of a patient in the Department of Dermatology of Faculty of Medicine, Charles University 100 years ago.

Proliferating cells, including stem cells require contact with the basement membrane, which is mediated predominantly by integrins forming contact of the hemidesmosomal type. Except type IV collagens, representatives of the laminin family and fibronectin, which are all directly recognized by integrins, oligo/polysaccharides including heparansulfate participate in the formation of the basement membrane. These carbohydrate components are recognized by the endogenous lectins, including the mannose-rich oligosaccharides of the laminin. They are also reactive for plant lectin – concavalin-A (Con-A) (Figure 2) (5). The carbohydrate–lectin interaction can modulate the protein–protein interaction performed by integrins and participates in the process of the control of the functional phenotype of proliferating cells (6). Detachment of epithelial cells induces, with several exceptions, a specific type of the programmed cell death, anoikis. The anoikis-resistant nucleated cells are (not surprisingly) leukocytes that are transported through the organism by blood/lymphatic vessels to the site of inflammation. Apart from these circulating cells of the immune system, adult (tissue) SCs including epidermal SCs (7, 8) can also be transported by the circulation and are anoikis resistant.

The basement membrane is clearly not only a simple passive scaffold of which the sole function is adherence. The basement membrane is reorganized during the life of the epithelium by activity of basal epithelial cells and underlying dermal/mucosal fibroblasts. The basement membrane represents an active fine-mesh screen that mediates adhesion and proliferation of epithelial cells and their support by nutritive substances, oxygen and signal molecules such hormones and growth factors (9, 10).

Epithelial–Mesenchymal Interaction in the Development of Adult Epithelium

Although as was mentioned above, the epithelial cells and fibroblasts are separated by the basement membrane, this barrier is not impermeable to oxygen, nutritive substances,

metabolites and signal molecules. The important data regarding the cooperation of both cell types of squamous epithelium, *i.e.* epithelial cells and fibroblasts was obtained from the developing skin and mucosa and its appendages such as hair, teeth, mammary gland and lungs. Morphologically, the epithelium is thickened at the epithelial bud around which the fibroblasts are concentrated (Figure 2). The extensive cross-talk between the epithelial and mesenchymal cells across the basement membrane is mediated *via* the production of specific growth factors (distinct members of bone morphogenetic factors, Wnt or hedgehog proteins), which is necessary for successful development (11-15). The similar well-orchestrated signaling machinery is active repeatedly during the cycle of the hair follicle from the anagen to catagen during its life. Fibroblasts harvested from distinct body surface areas such as the palm/planta control the ectopic expression of specific keratins (K9) in keratinocytes obtained from the breast, originally K9-negative (16).

Normal and Cancer SCs

The majority of tissues including squamous cell epithelium contain SCs. As mentioned above, the epidermal SCs are located in the bulge region of the outer root sheath and in the basal layer of epithelium. Their membrane is equipped by special proteins responsible for the active efflux of xenobiotics out of the cytoplasm, so protecting the cells from the toxic damage (side cell population) (17). It should be noted here that the special microenvironment (so-called niche) is responsible for the maintenance of SC properties and preservation against their differentiation (18, 19).

An abundance of data indicate that leukemias and solid tumors also contain a pool of cells sharing SC properties. These cells were initially defined during transplantation experiments where only a small portion of cells of the tumor mass is responsible for the tumor successful transfer of

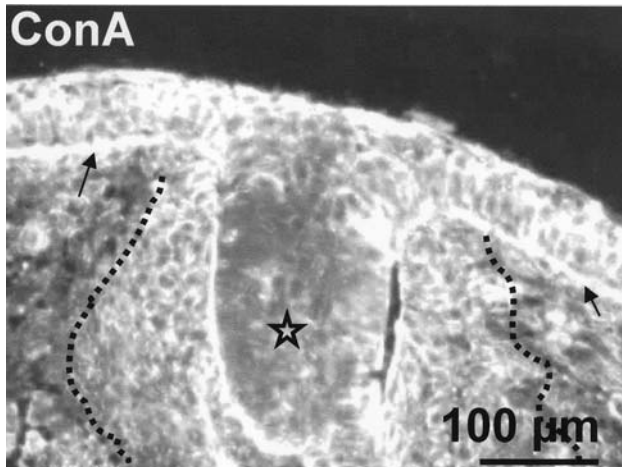


Figure 2. Developing hair follicle from skin of 14-day-old mouse embryo. The carbohydrates reactive for plant endogenous lectin-concavalin-A (ConA) exhibit a distinct signal. The high signal is present in the basement membrane separating the epithelium from underlying mesenchyma (arrows) including the epithelium-thickening bud (asterisk). Mesenchyme cell accumulation around the epithelium bud (asterisk) is marked by a dashed line.

tumor to the host (20, 21). Indirectly, the cells with phenotypic signature of tissue SCs were described in human and animal carcinomas, such as tumors of breast, skin, mucosa and prostate (4, 22-25). Moreover, both cell populations *i.e.* adult SCs and some of cancer cells are resistant to anoikis (7, 26). Because their other properties are also similar to that of normal adult SCs *i.e.* slow cell cycle, unlimited proliferation rate and membrane proteins responsible for exclusion of xenobiotics, their existence may be clinically very important. Their destruction by chemo/radiotherapy is thus not easy and their unique properties can cause clinically important phenomena such as minimal residual disease and multidrug resistance (4). These cells represent an ideal target for anticancer therapy. Unfortunately, no specific markers of these cells are known and, therefore, their specific targeting is not yet possible.

If we accept the existence of putative cancer SCs, it is possible to hypothesize the likely role of their niche on 'successful' growth of tumor and their metastasis.

Tumor Stroma as the Niche for Cancer SCs

Almost 100 years ago, the British surgeon Stephen Paget postulated the hypothesis that cancer cells need a special environment in a similar way to seeds needing fruitful soil (27). Pathologists know that the connective tissue surrounding the epithelial tumor cells buds, so-called tumor stroma, is usually different from the mesenchymal component of normal tissue. The tumor stroma is a complex tissue composed of

Table I. Examples of bioactive molecules in alphabetical order produced by cancer-associated fibroblasts influencing the biological properties of tumor (adapted from references 36, 39 and 56-60).

Molecule	Usual abbreviation
A disintegrin and metalloproteinase	(ADAM-S9)
Amphiregulin	
Bone morphogenetic factor 4	BMP-4
Cyklooxygenase products	
Fibroblast growth factor 7 and 10	FGF-7, FGF-10
Gremlin 1	
Macrophage-colony stimulating factor	GM-CSF
Insulin like growth factor 1 and 2	IGF-1, IGF-2
Interleukin 6 and 8	IL-6, IL-8
Monocyte chemoattractant protein 1	MCP-1
Matrix metalloproteinase 1, 2 and 9	MMP-1, MMP-2, MMP-9
Regulated on activation normal T-cell expressed and secreted Protein S100 A4	
Stromal cell-derived factor 1	SDF-1
Scatter factor/hepatocyte growth factor	SC/HGF
Secreted frizzled-related protein 1	SFRP1
Syndecan-1	
Tenascin-C and W	
Transforming growth factor β 1	TGF- β 1
Vascular endothelial growth factor	VEGF

fibroblasts (and extracellular matrix produced by these cells), capillaries (morphologically distinct) and different types of leukocytes (Figure 3A, B). The classical view of the tumor stroma predicted its role only for the adhesion, support and nutrition of cancer cells. However, the contemporary view suggests that the tumor stroma can influence the biological properties of tumor, mainly its local aggressivity and ability to metastasize (28-30). There are certain similarities concerning the arrangements of tumor stroma and connective tissue component of wounds (31). For example, we observed a remarkably strong expression of adhesion/growth regulatory lectin galectin-1 in the dermis of wounded skin, and stroma of basal (BCC), squamous cell carcinoma (SCC) and also in the dermis of psoriatic plaque (32-36). Another connecting feature of wound with tumor is the existence of a special population of fibroblasts, some of which exhibit smooth muscle actin (the myofibroblasts) (37). These cells originate predominantly from the local mesenchyme by the activity of transforming growth factor β (TGF- β) (Figure 3C, D) (38). Fibroblasts/myofibroblasts participate in defect closure by wound contraction (39). However, they are also known to be potent producers of extracellular matrix and of numerous cytokines/chemokines directly regulating the activation of epithelial cell proliferation and migration (Table I). The tumor stromal fibroblasts/myofibroblasts can participate in the control of the activation of cancer cells (including cancer SCs) (37, 40).

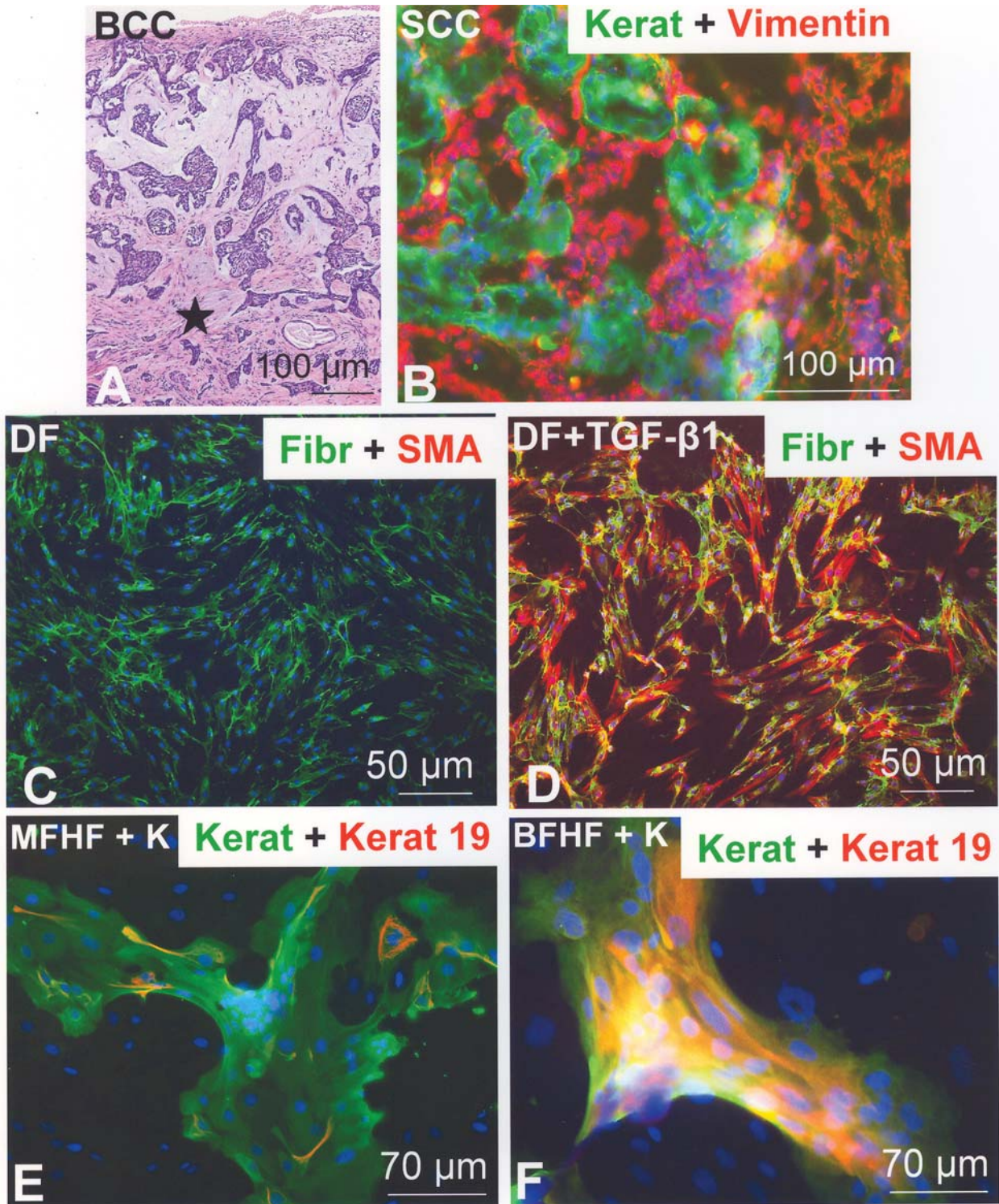


Figure 3. Advanced basal cell carcinoma (BCC) stained by hematoxylin and eosin (A) is characterized by the desmoplastic appearance of tumor stroma (asterisk). Cells of squamous cell carcinoma (SCC) of larynx (B) positive for pankeratin (kerat) (green signal) are surrounded by frequent stromal elements positive for vimentin (red signal). Dermal fibroblasts (DF) produce fibronectin (green signal) (C). The application of TGF- β 1 significantly induces transformation of fibroblasts to myfibroblasts exhibiting α -smooth muscle actin (SMA) (red signal) (D). Production of fibronectin is preserved. Normal epidermal keratinocytes (K) were co-cultured with fibroblasts prepared from malignant fibrous histiocyctoma (MFHF) (E) and from benign fibrous histiocyctoma (BFHF) (F) under identical conditions. The difference in expression of keratin 19 (red signal) is clearly visible. Nuclei are counterstained by 4, 6-diamidino-2-phenylindole (DAPI). Bar is 100 μ m (A, B), 50 μ m (C, D) and 70 μ m (E, F) respectively.

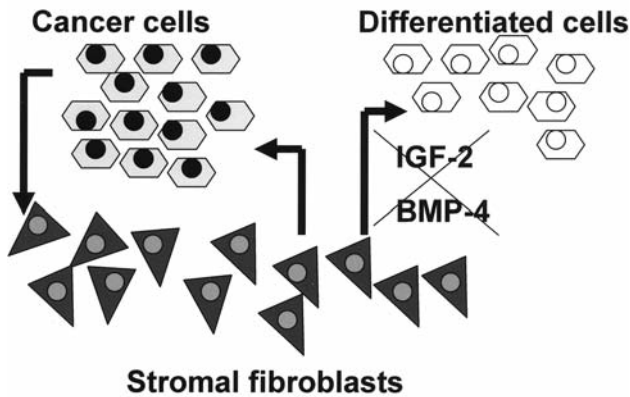


Figure 4. Bidirectional mutual interaction between cancer epithelial cells and stromal fibroblasts influences formation of permissive cancer microenvironment. Blocking of molecules participating in this interaction, such as IGF-2 and BPM-4 (60) by antibodies significantly affects phenotype of epithelial cells to exhibit markers of advanced differentiation. Therapeutic potential of cancer microenvironment modulation is possible.

We have isolated cancer stromal fibroblasts from BCC of the skin and SCC of the tongue as previously published elsewhere (34, 41). Co-culturing these fibroblasts with normal human keratinocytes, we observed their strong impact on the phenotype of normal human keratinocytes acquiring a phenotype similar to that of tumor epithelium or the epidermal SC pool. BCC stromal fibroblasts induced expression of K19 in normal keratinocytes. This type of intermediate filament is expressed by the bulge epidermal SCs and more than 50% of BCCs exhibit cells positive for this keratin (8). This observation was supported by induction of the nuclear expression of nucleostemin and binding sites for galectin-1 that also indicate the low level of the differentiation of co-cultured keratinocytes. The observation of the expression of BCC marker (Ber-Ep4) in normal keratinocytes co-cultured with BCC fibroblasts underlines the role of stromal elements in normal epithelium.

To verify this observation, we employed another type of skin tumor. Dermatofibroma (benign fibrous histiocytoma) is a common fibrohistiocytic skin tumor of mesenchymal origin where the pool of tumor cells has properties of atypical histiocytes. Curiously, the epidermis overlying the tumor nodule (that is present in dermis) is hyperplastic and similar to BCC. The fibroblasts isolated from this type of tumor are very similar to the fibroblasts isolated from the BCC in relation to their biological activity, where they also induce expression of K19 in co-cultured normal keratinocytes. On the contrary, malignant fibrous histiocytoma, another soft tissue tumor of dermal origin is not associated with hyperplasia of overlying epidermis. In concordance with this experience from histopathology, no response in the normal

human keratinocytes was observed during *in vitro* co-cultivation experiments (Figure 3E, F) (42, 43).

The phenotypic changes of normal keratinocytes under the influence of co-cultured fibroblasts prepared from the stroma of SCC were stronger in comparison with that of the fibroblasts from the BCC/dermatofibroma (41). In addition to the induction of expression of K19, we also detected cells exhibiting K8. This intermediate filament is never expressed by normal squamous epithelium postnatally, but it is present in SCC where it is an indicator of poor prognosis for the patient (44). Many normal keratinocytes co-cultured with SCC stromal fibroblasts also coexpressed keratin with vimentin. These cells also exhibited nuclear presence of transcription factor SNAIL that is known to participate in the control of epithelial-mesenchymal transition (EMT) (45). This observation suggests EMT in keratinocytes co-cultured with SCC stromal fibroblasts. This result seems to be functionally important because EMT participates in embryogenesis, wound healing and in the process of metastasis (46-48).

Mesenchymal bone marrow stromal cells are able to acquire properties of cancer-associated fibroblasts (49). Similarly, the cells formed by the EMT capture properties of mesenchymal SCs (50). Therefore it is probable that some stromal fibroblasts can originate directly in the cancer epithelium. This possibility was proposed for breast cancer (51). Mechanisms of how the cancer-associated fibroblasts originate are summarized in Table II.

The majority of SCCs of uterine cervix and many head and neck SCCs are induced by human *Papillomavirus 16* (HPV16) (53). The genetically engineered epithelial cells transfected by protooncogenes *E6* and *E7* of HPV16 acquire morphological properties of mesenchymal cells (fibroblasts). Hence they can represent the mesenchymal cells arising from cancer epithelium by the EMT. These cells share the genetic alteration identical to that of the original tumor population, however their morphology and functional properties are strikingly different. We co-cultured these transfected cells of fibroblast-like morphology together with normal keratinocytes. Their influence on the normal keratinocytes was profound and very similar to the action of fibroblasts prepared from the stroma of SCC. This indicates that the tumor epithelium can be the source of bioactive stromal fibroblasts. This hypothesis needs verification by mutation analysis, because when the stromal fibroblasts should be originated in tumor stroma, their mutation should be the same as that, in cancer cells. Unfortunately, analyzing the tumors from clinical practice produces data that are controversial (54). Moreover, the inclusion of cancer cells migrating through the stroma in the course of metastasis can be a critical objection to this hypothesis because it is not possible to distinguish them from stromal elements formed by the epithelial-mesenchymal interaction.

When stromal fibroblasts are maintained *in vitro* for a long time their biological activity is preserved for at least 2

Table II. Examples of the most probable mechanisms of cancer-associated fibroblast formation (adapted from references 37, 49-52).

Source of cancer-associated fibroblasts	Mechanism
Local mesenchyme	Paracrine activation of local mesenchyme by cancer cells and/or inflammatory cells
Cancer epithelium	Epithelial-mesenchymal transition
Macrophage Myoepithelial cell Endothelial cell Pericyte Smooth muscle cell Hepatic stellate cell Adipocyte	Transition to cancer-associated fibroblasts
Mesenchymal stem cell	Homing to site of tumor and transition to cancer-associated fibroblasts

months, although these cells are isolated from the influence of tumor epithelium (34, 41). This property can be caused principally by two mechanisms. In addition to direct genetic alteration, epigenetic changes could also be responsible for this phenomenon. The data summarized by Polyak and co-workers indicate that epigenetics can play an important role in the biology of cancer stromal fibroblasts (55).

Although, the data demonstrated the effect of cancer stromal fibroblasts/myofibroblasts on the biology of epithelial cells, the unanswered question is whether the contact of cell populations *i.e.* stromal elements and the epithelium is necessary. In order to address this question, we co-cultured epithelial cells with stromal fibroblasts directly on coverslips and in a transwell system. Both cell types were separated by a microporous membrane, avoiding their direct contact, but enabling the free movement of cultivation medium containing soluble molecules including growth/regulatory factors. It should be noted here that the activity of fibroblasts relative to that of co-cultured epithelium was lower, but principally it was preserved (34, 41). This result indicates the important role of soluble proteins (cytokines/chemokines) produced by stromal fibroblasts/myofibroblasts (Table I) in mesenchymal-epithelial interaction in tumors, where the examples are shown here (56-60). The remarkable role of these regulatory factors is not so surprising because, as was shown above, both cell populations, *i.e.* epithelium and underlying mesenchyme, are separated by a permeable basement membrane under normal physiological conditions, where the epithelial cells are adapted to recognize these bioactive molecules. Malignant tumors are characterized by invasion of tumor cells across the basement membrane that characterizes the transition of the so-called carcinoma *in situ* to the invasive form. However, as was depicted in the case of head and neck SCC, the basement membrane, frequently incomplete, surrounds a substantial proportion of nodules of

tumor epithelium, usually, except the site of invasive front of tumor with actively migrating cells (61). This data suggests that the epithelium is adapted for the signaling by active proteins/peptides across the permeable membrane even in cancer.

On the other hand, the opposite role of the niche as presented above was clearly demonstrated by experiments where the cells with a high tumorigenic potential were influenced by the embryonic microenvironment, minimizing their tumor-forming activity and malignant phenotype (62).

Conclusion and Future Perspectives

This review article has demonstrated that the microenvironment of tumor stroma participates in the control of the biological properties of cancer, including tumors arising from squamous epithelia and it can be considered as the niche of cancer SC. Stromal fibroblasts/myofibroblasts represent a pool of cells with remarkable biological activity for the production of extracellular matrix and bioactive substances, controlling the differentiation of epithelial cells, their EMT and migration. This indicates that the detailed molecular analysis of tumor stroma must be undertaken to characterize the biological properties of tumor of specific patients to prepare tailor-made individualized therapy. The stromal elements and their products represent potential targets of a new type of adjuvant anticancer therapy: for example, the cytokines/chemokines such as insulin-like growth factor 2 (IGF-2) or bone morphogenetic factor 4 (BMP-4) important for tumor progression can be neutralized by specific monoclonal humanized antibodies or their receptor activity can be minimized by antagonists (Figure 4) (60, 63). The new horizons of tumor stroma research are also apparent in regenerative medicine, as it has been demonstrated that normal epithelial cells switch their phenotype to SC-like when they are influenced by tumor stroma fibroblasts.

Acknowledgements

Some of the results presented in this review were obtained in research projects supported by the Ministry of Education, Youth and Sport of the Czech Republic (project no. MSM0021620806, 1M0538 and NPVII 2B06106), Grant Agency of the Czech Republic (project no. 304/08/P175) and European Commission (contract no. MRTN-CT-2005-019561).

References

- Smetana K Jr, Plzák J, Dvořánková B and Holíková Z: Functional consequences of the glyco-phenotype of squamous epithelia – practical employment. *Folia Biol* 49: 118-127, 2003.
- Bickenbach JR and Grinnell KL: Epidermal stem cells: interactions in developmental environments. *Differentiation* 72: 371-380, 2004.
- Blanpain C and Fuchs E: Epidermal stem cells of the skin. *Ann Rev Cell Dev Biol* 22: 339-373, 2006.
- Motlík J, Klíma J, Dvořánková B and Smetana K Jr: Porcine epidermal stem cells as a biomedical model for wound healing and normal/malignant epithelial cell propagation. *Theriogenol*, 67: 105-111, 2007.
- Labský J, Dvořánková B, Smetana K Jr, Holíková Z, Brož L and Gabius H-J: Mannosides as crucial part of bioactive supports for cultivation of human epidermal keratinocytes without feeder cells. *Biomaterials* 24: 863-872, 2003.
- Fischer C, Sanchez-Ruderisch H, Welzel M, Wiedenmann B, Sakai T, André S, Gabius H-J, Khachigian L, Detjen KM and Rosewicz S: Galectin-1 interacts with the $\alpha 5\beta 1$ fibronectin receptor to restrict carcinoma cell growth *via* induction of *p21* and *p27*. *J Biol Chem* 280: 37266-37277, 2005.
- Chiarugi P and Giannoni E: Anoikis: a necessary death program for anchorage-dependent cells. *Biochem Pharmacol* 76: 1352-1364, 2008.
- Dvořánková B, Smetana K Jr, Chovanec M, Lacina L, Štork J, Plzáková Z, Galovičová M and Gabius H-J: Transient expression of keratin 19 is induced in originally negative interfollicular epidermal cells by adhesion of suspended cells. *Int J Mol Med* 16: 525-531, 2005.
- Kamer JM: Basement membranes. *WormBook*, September 1: 1-15, 2005.
- Breitkreutz D, Mirancea N and Nischt R: Basement membranes in skin: unique matrix structures with diverse functions? *Histochem Cell Biol* 132: 1-10, 2009.
- Hatsell S and Frost AR: Hedgehog signaling in mammary gland development and breast cancer. *J Mammary Gland Biol Neopl* 12: 163-173, 2007.
- De Langhe SP and Reynolds SD: Wnt signaling in lung organogenesis. *Organogenesis* 4: 100-108, 2008.
- Salazar-Ciudad IT: Tooth morphogenesis *in vivo*, *in vitro*, and *in silico*. *Curr Top Dev Biol* 81: 341-371, 2008.
- Yu BD, Mukhopadhyay A and Wong C: Skin and hair: models for exploring organ regeneration. *Hum Mol Gen* 17: R54-59, 2008.
- Schneider MR, Schmidt-Ullrich R and Paus R: The hair follicle as a dynamic miniorgan. *Cur Biol* 19: R132-142, 2009.
- Yamaguchi Y, Itami S, Tarutani M, Hosokawa K, Miura H and Yoshikawa K: Regulation of keratin 9 in nonpalmoplantar keratinocytes by palmoplantar fibroblasts through epithelial-mesenchymal interactions. *J Invest Dermatol* 112: 483-488, 1999.
- Yano S, Ito Y, Fujimoto M, Hamazaki TS, Tamaki K and Okochi H: Characterization and localization of side population cells in mouse skin. *Stem Cells* 23: 834-841, 2005.
- Ambler CA and Määttä A: Epidermal stem cells: location, potential and contribution to cancer. *J Pathol* 217: 206-216, 2009.
- Walker MR, Patel KK and Stappenbeck TS: The stem cell niche. *J Path* 217: 169-180, 2009.
- Park CH, Bergsagel DE and McCulloch EA: Mouse myeloma tumour stem cells: a primary cell culture assay. *J Natl Cancer Inst* 46: 411-422, 1971.
- Hamburger AW and Salmon SE: Primary bioassay of human tumour stem cells. *Science* 197: 461-463, 1977.
- Sell S: Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol* 51: 1-28, 2004.
- Chovanec M, Smetana K Jr, Betka J, Plzák J, Brabec J, Moya-Alvarez V, André S, Kodet R and Gabius H-J: Correlation of expression of nuclear proteins pKi-67 and p63 with lectin histochemical features in head and neck squamous cell cancer. *Int J Oncol* 27: 409-415, 2005.
- Glinsky GV: Stem cell origin of death-from-cancer phenotypes of human prostate and breast cancers. *Stem Cell Rev* 3: 79-93, 2007.
- Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloustain-Qimron N, Yao J, Nikolskaya T, Serebryskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS and Polyak K: Molecular definition of breast tumor heterogeneity. *Cancer Cell* 11: 259-273, 2007.
- Grossmann J: Molecular mechanisms of 'detachment-induced apoptosis-anoikis'. *Apoptosis* 7: 247-260, 2002.
- Fidler IJ: The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nature Rev Cancer* 3: 453-458, 2003.
- Barcellos-Hoff MH and Ravani SA: Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Res* 60: 1254-1260, 2000.
- Lorusso G and Rüegg C: The tumor microenvironment and its contribution to tumor evolution toward metastasis. *Histochem Cell Biol* 130: 1091-1103, 2008.
- Casey T, Bond J, Tighe S, Hunter T, Lintault L, Patel O, Eneman J, Crocker A, White J, Tessitore J, Stanley M, Harlow S, Weaver D, Muss H and Plaut K: Molecular signatures suggest a major role for stromal cells in development of invasive breast cancer. *Breast Cancer Res Treat* 114: 47-62, 2009.
- Dvorak HF: Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *New Engl J Med* 315: 1650-1659, 1986.
- Lacina L, Plzáková Z, Smetana K Jr, Štork J, Kaltner H and André S: Glyco-phenotype of psoriatic skin. *Folia Biol* 52: 10-15, 2006.
- Čada Z, Bouček J, Dvořánková B, Chovanec M, Plzák J, Kodet R, Betka J, Pinot GL, Gabius H-J and Smetana K Jr: Nucleostemin expression in squamous cell carcinoma of the head and neck. *Anticancer Res* 27: 3279-3284, 2007.
- Lacina L, Smetana K Jr, Dvořánková B, Pytlík R, Kideryová L, Kucerová L, Plzáková Z, Štork J, Gabius H-J and André S: Stromal fibroblasts from basal cell carcinoma affect phenotype of normal keratinocytes. *Br J Dermatol* 156: 819-829, 2007.
- Čada Z, Smetana K Jr, Lacina L, Plzáková Z, Štork J, Kaltner H, Russwurm R, Lensch M, André S and Gabius H-J: Immunohistochemical fingerprinting of the network of seven adhesion/growth-regulatory lectins in human skin and detection of distinct tumour-associated alterations. *Folia Biol* 55: 145-152, 2009.

- 36 Saussez S, Decaestecker C, Cludts S, P. Ernoux, Chevalier D, Smetana K Jr, André S, Leroy X and Gabius H-J: Adhesion/growth-regulatory tissue lectin galectin-1 in relation to angiogenesis/lymphocyte infiltration and prognostic relevance of stromal up-regulation in laryngeal carcinomas. *Anticancer Res* 29: 59-65, 2009.
- 37 De Wever O, Demetter P, Mareel M and Bradle M: Stromal myofibroblasts are drivers of invasive cancer growth. *Int J Cancer* 123: 2229-2238, 2008.
- 38 Denis H, Derycke L, Hendrix A, Westbroek W, Gheldof A, Narine K, Pauwels P, Gespach C, Bracke M and De Wever O: Differential impact of TGF- β and EGF on fibroblast differentiation and invasion reciprocally promotes colon cancer cell invasion. *Cancer Let* 266: 263-274, 2008.
- 39 Hinz B: Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol* 127: 526-537, 2007.
- 40 De Wever O, Nguyen QD, Van Hoorde L, Bracke M, Bruyneel E, Gespach C and Mareel M: Tenascin-C and SF/HGF produced by myofibroblasts *in vitro* provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. *FASEB J* 18: 1016-1028, 2004.
- 41 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 and Gabius H-J: 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, 2007.
- 42 Han K-H, Huh C-H and Cho K-H: Proliferation and differentiation of the keratinocytes in hyperplastic epidermis overlying dermatofibroma: immunohistochemical characterization. *Am J Dermatopathol* 23: 90-98, 2001.
- 43 Kideryová L, Lacina L, Dvořánková B, Štork J, Čada Z, Szabo P, André S, Kaltner H, Gabius H-J and Smetana K Jr: Phenotypic characterization of human keratinocytes in coculture reveals differential effects of fibroblasts from benign fibrous histiocytoma (dermatofibroma) as compared to cells from its malignant form and to normal fibroblasts. *J Dermatol Sci* 55: 18-26, 2009.
- 44 Fillies T, Werkmeister R, Packeisen J, Brandt B, Morin P, Weingart D, Joos U and Berger H: Cytokeratin 8/18 expression indicates a poor prognosis in squamous cell carcinomas of the oral cavity. *BMC Cancer* 6: 10, 2006.
- 45 Thiery JP and Sleeman JP: Complex networks orchestrate epithelial-mesenchymal transitions. *Nature Rev Mol Cell Biol* 7: 131-142, 2006.
- 46 Tucker RP: Neural crest cells: a model for invasive behavior. *Int J Biochem Cell Biol* 36: 173-177, 2004.
- 47 Savagner P, Kusewitt DF, Carver EA, Magnino F, Choi C, Gridley T and Hudson LG: Developmental transcription factor slug is required for re-epithelialization by adult keratinocytes. *J Cell* 202: 858-866, 2005.
- 48 Moustakas A and Heldin CH: Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. *Cancer Sci* 98: 1512-1520, 2007.
- 49 Mishra PJ, Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW and Banerjee D: Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer Res* 68: 4331-4339, 2008.
- 50 Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J and Weinberg RA: The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 133: 704-715, 2008.
- 51 Petersen OW, Nielsen HL, Gudjonsson T, Villadsen R, Rank F, Niebuhr E, Bissell MJ and Rønnov-Jessen L: Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. *Am J Pathol* 162: 391-402, 2003.
- 52 Haviv I, Polyak K, Qiu W, Hu M, Campbell I: Origin of carcinoma-associated fibroblasts. *Cell Cycle* 8: 589-595, 2009.
- 53 Smetana K Jr, Dvořánková B, Lacina L, Čada Z and Vonka V: Human hair follicle and interfollicular keratinocyte reactivity to mouse HPV16-transformed cells: an *in vitro* study. *Oncol Rep* 20: 75-80, 2008.
- 54 Weinberg RA: Coevolution in the tumor microenvironment. *Nature Genet* 40: 494-495, 2008.
- 55 Polyak K, Haviv I and Campbell IG: Coevolution of tumor cells and their microenvironment. *Trends Genet* 25: 30-38, 2008.
- 56 Dhawan P and Richmond A: Role of CXCL1 in tumorigenesis of melanoma. *J Leukocyte Biol* 72: 9-18, 2002.
- 57 Aikawa T, Gunn J, Spong SM, Klaus SJ and Korc M: Connective tissue growth factor-specific attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. *Mol Cancer Ther* 5: 1108-1116, 2006.
- 58 Sneddon JB, Zhen HH, Montgomery K, van de Rijn M, Tward AD, West R, Gladstone H, Chang HY, Morganroth GS, Oro AE and Brown PO: Bone morphogenetic protein antagonist gremlin 1 is widely expressed by cancer-associated stromal cells and can promote tumor cell proliferation. *Proc Natl Acad Sci USA* 103: 14842-14847, 2006.
- 59 Wang D, Wang H, Brown J, Daikoku T, Ning W, Shi Q, Richmond A, Strieter R, Dey SK and DuBois RN: CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. *J Expl Med* 203: 941-951, 2006.
- 60 Strnad H, Lacina L, Kolář M, Čada Z, Vlček Č, Dvořánková B, Betka J, Plzák J, Chovanec M, Šáchorová J, Valach J, Urbanová M and Smetana K Jr: Head and neck squamous cancer fibroblasts produce growth factors influencing phenotype of normal human keratinocytes. *Histochem Cell Biol*, 2009.
- 61 Čada Z, Chovanec M, Smetana K Jr, Betka J, Lacina L, Plzák J, Kodet R, Štork J, Lensch M, Kaltner H, André S and Gabius H-J: Galectin-7: will the lectin's activity establish clinical correlations in head and neck squamous cell and basal cell carcinomas? *Histol Histopathol* 24: 41-48, 2009.
- 62 Postovit L-M, Margaryan NV, Sektor EA, Kirshmann DA, Lapavky Y, Wheaton WW, Abott DA, Sektor REB and Hendrix MJC: Human embryonic stem cell microenvironment suppresses the tumorigenic phenotype of aggressive cancer cells. *Proc Natl Acad Sci USA* 105: 4329-4334, 2009.
- 63 Kenny PA, Lee GY and Bissell MJ: Targeting the tumor microenvironment. *Front Biosci* 12: 3468-3474, 2007.

Received August 3, 2009

Revised December 23, 2009

Accepted December 29, 2009