

Chapter 2

Functional Diversity of Fibroblasts

H. Peter Rodemann and Hans-Oliver Rennekampff

2.1 Introduction

Solid tumours are multi-cellular tissues comprised of tumour cells and stromal cells, including fibroblasts, endothelial cells and inflammatory cells. When a cancer cell metastasizes, it first will be exposed to cancer associated fibroblasts in the immediate tumour microenvironment and subsequently to normal fibroblasts as it traverses the underlying connective tissue on its way to the bloodstream. So far, the interactions of tumour cells with stromal fibroblasts influence tumour biology by mechanisms that are not yet fully understood. It is known that cells of the tumour parenchyma and stroma are in extensive crosstalk, and the composition of the stroma and the nature of tumour stromal interactions change over time with tumour progression (Beacham and Cukierman 2005; Proia et al. 2005). The tumour-stroma crosstalk markedly influences not only tumour growth by modifying and controlling angiogenesis, suppressing or subverting immune responses of the host, but also by modulating extracellular matrix composition, and secreting factors which in turn stimulate cells to further alter cell physiology as well as the cellular and acellular composition of the tumour microenvironment (Stuelten et al. 2010; Olumi et al. 1999; Liotta and Kohn 2001).

Fibroblasts as ubiquitous stromal cells are accessory cells which influence other neighbouring cell types through secretion of cytokines as well as growth and differentiation factor factors (Baglolle et al. 2006; Micke and Ostman 2005). While in the early phase fibroblasts can have tumour suppressing activity the phenotype of fibroblasts can alter to a tumour promoting state as carcinogenesis progresses (Proia et al. 2005). This phenotypic switch does occur in two stages in which a reversible “primed” fibroblasts type is generated first followed the manifestation of an irreversible phenotype of fibroblasts which present tumour-promoting properties

H. P. Rodemann (✉)

Division of Radiobiology & Molecular Environmental Research,
Department of Radiation Oncology, Eberhard Karls University Tübingen,
Röntgenweg 11, 72076 Tübingen, Germany
e-mail: hans-peter.rodemann@uni-tuebingen.de

(Beacham and Cukierman 2005). These so-called cancer associated fibroblasts (CAF) have properties distinct from normal fibroblasts and actively promote tumorigenesis as it has been described for example for prostate and breast cancer (Olumo et al. 1999; Orimo et al. 2005).

The topic of cancer associated fibroblasts, however, is discussed in separate chapters of this book. In the following various aspects of the functional diversity of normal not cancer associated fibroblast will be discussed as the capacity of fibroblasts to produce and organize the extracellular matrix and to communicate with other cell systems makes them a central component of tissue biology.

2.2 Fibroblast Phenotypes

Fibroblasts from different anatomical regions display characteristic phenotypes. Early work on the heterogeneity of fibroblasts performed by Castor and co-workers (Castor et al. 1962) demonstrated metabolic differences between mesothelial fibroblasts, fibroblasts of the skin, articular tissues and periosteum. Studies by Chang et al. (2002) and Rinn et al. (2006, 2008) characterized the gene expression profile of fibroblasts derived from different anatomical regions of the body with cDNA microarray technology. These authors were able to show a site specific gene expression pattern with a striking division into: anterior-posterior, proximal-distal and dermal-non dermal. In addition they demonstrated diversity in topographic expression for genes involved in extracellular matrix synthesis, growth and differentiation, cell migration as well as genes involved in genetic syndromes. Work by Chipev and Simon (2002) indicated that fibroblasts from different body sites differ in size, with palmar fibroblasts being smaller than non-glabrous derived fibroblasts. In addition growth kinetics and TGF (Transforming Growth Factor) β 1 Receptor II expression as well as the ability to contract collagen lattices were found to differ with palmoplantar skin derived fibroblasts having lower receptor levels and an increased mitotic rate. The authors speculated that this regional diversity may in part account for localized susceptibility to disease manifestation like scarring and keloid formation. Recent work on oral mucosal fibroblasts has demonstrated the differences in the capacity of these fibroblasts to reorganize collagen lattices by an increased matrix metalloproteinase (MMP)-2 expression as compared to skin derived fibroblasts (Stephens et al. 2001). Furthermore, it was shown that oral mucosa derived fibroblasts proliferated more rapidly and had a higher capacity for cell doublings. A functional correlation was linked to the fact that cultured oral fibroblasts secret more hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) than skin fibroblasts (Gron et al. 2002). It was suggested that both effects may contribute to a 'fetal' wound healing phenotype of oral fibroblasts.

Beside the differences in fibroblasts from different anatomical sites fibroblasts separated from a single tissue are similarly not composed of a homogeneous population but rather consist of subsets of different fibroblasts. Sorrell and Caplan (2004) have reviewed differences between papillary fibroblasts, which reside in the

superficial dermis and reticular fibroblasts which are located in the deeper dermis, highlighting the difference in matrix molecule production from these two distinct cell populations. Versican is produced at low levels by papillary fibroblasts while reticular fibroblasts produce high levels of this molecule. In contrast, decorin is produced in high levels by papillary fibroblast but only in small amounts by reticular fibroblasts. Collagen type I and collagen type III production and the subsequent ratio of these two dermal collagens were not found to differ between these populations of fibroblasts.

Studies from the laboratory of the author also point toward the existence of different morphological and biochemical subsets of fibroblasts within the same tissue. Two major populations of fibroblasts have been detected in the dermis, lung and kidney termed mitotically active, i.e. replicative progenitor fibroblasts (MF) and irreversible postmitotic fibrocytes (PMF). These two subsets have subsequently been classified by cytomorphological and biochemical characteristics (Bayreuther et al. 1988; Rodemann 1989; Rodemann and Mueller 1990; Rodemann and Bamberg 1995; Lara et al. 1996; Rodemann et al. 1996; Burger et al. 1998; Herskind et al. 1998; Hakenjos et al. 2000). Based on in vitro and ex vivo-in vitro experiments it was shown that fibroblasts of human, rat and mouse skin but also lung as well as renal fibroblasts differentiate in vivo and in vitro along a lineage of replicative progenitor fibroblast cell types and non replicative, postmitotic functional fibrocytes of man, rat and mouse (Mollenhauer and Bayreuther 1986; Bayreuther et al. 1988; Rodemann et al. 1991). According to their cytomorphology (Fig. 2.1), replicative potential and ability to synthesize specific cytokines and growth factors, like TGF- β and Keratinocyte Growth Factor (KGF), MF-progenitor fibroblasts can be further classified into the cell types MF I, MF II, and MF III (Rodemann 1989; Herskind et al. 1998; Burger et al. 1998; Hakenjos et al. 2000). Subcloning experiments revealed that these MF-cell types differentiate along the lineage MF I, MF II,

Parameter	MFI	MFII	MFIII	PMF
Cell division potential	+++	++	+	-
SA- β -Gal expression	-	(+)	+	+++
Total collagen synthesis	(+)	+	+	+++
KGF-production	(+)	+	++	+++
TGF β 1-production	+	+	+++	+++

Fig. 2.1 Differentiation lineage, marker and collagen expression, as well as growth factor production profile of progenitor fibroblasts and postmitotic fibrocytes. The differentiation lineage of progenitor fibroblasts (MF) and postmitotic fibrocytes (PMF) along the sequence MFI-MFII-MFIII-PMF cell types was analysed on the basis of cell biological and biochemical parameters. Quantitative and qualitative differences could be demonstrated for the parameter cell division potential, SA- β -galactosidase (SA- β -Gal) expression, total collagen synthesis, KGF and TGF β 1 production. A relative indicator system (- negative; (+) very low; + low; ++ moderate; +++ high) is chosen for cell type specific comparison of these parameters

MF III, before differentiation into irreversible postmitotic fibrocytes (PMF) occurs (Bayreuther et al. 1988, 1989; Rodemann et al. 1996). MF I-type progenitor fibroblasts have the highest replicative capacity. MFI progenitor fibroblasts of human origin can go through about cell divisions before differentiating into MF II type cells. This cell type can divide approximately 15–20 times before differentiating into cell type MF III which has the potential for only 5–8 further division cycles before differentiating spontaneously into irreversible postmitotic fibrocytes (Rodemann et al. 1989). Based on its biochemical activity the latter cell type reflects the functional cell type of the fibroblast-fibrocyte cell system. Per cell it produces about 5–8 times more total collagen as compared to progenitor fibroblasts and provides the correct ratio of interstitial collagens type I, III, and V needed for homeostasis and maintenance of interstitial tissues (Rodemann et al. 1989). The cellbiological and biochemical parameters of progenitor fibroblasts and postmitotic fibrocytes are summarized Fig. 2.1.

As reported earlier for human skin (Bayreuther et al. 1988; Rodemann 1993; Herskind et al. 1998) as well as rat lung (Hakenjos et al. 2000) a constant ratio in the in vivo proportion of progenitor fibroblasts and functional postmitotic fibrocytes of 2:1 exists and seems to be independent of the age of the donor. Thus it can be assumed that biochemical homeostasis of the dermis as well as of interstitial tissues in various organs depends on a correct ratio of progenitor fibroblasts and functional postmitotic fibrocytes. Disturbance of this ratio by exogenous or endogenous factors may lead to an altered cellular homeostasis in the affected tissue and can result in pathologic tissue remodelling, similar to that observed in renal, lung and skin fibrosis (Rodemann and Bamberg 1995; Rodemann et al. 1996; Burger et al. 1998; Herskind et al. 1998; Rodemann and Mueller 1990; Rodemann et al. 1991; Hakenjos et al. 2000; von Pfeil et al. 2002).

Under in vitro conditions, progenitor fibroblasts and functional postmitotic fibrocytes can be classified according to the specific expression of the senescence activated- β -Galactosidase activity (SA- β -Gal), which is also known as pH6-activity of β -galactosidase. Postmitotic fibrocytes show a strong expression of SA- β -Gal activity whereas MF-progenitor fibroblasts do not (Hakenjos et al. 2000). The expression of this enzyme activity has been used in attempts to identify cellular ageing processes in fibroblasts cultures (Dimri et al. 1995). The expression of pH6- β -galactosidase activity could only be demonstrated in fibroblasts which have undergone replicative senescence, i.e. in irreversible postmitotic cells (Dimri et al. 1995). These fibroblast populations are predominantly composed by the functional and irreversible postmitotic fibrocytes (Herskind et al. 1998; Hakenjos et al. 2000) (for summary see Fig. 2.1). Decreased proliferative potential of fibroblasts has also been noticed in a stress-induced senescence-like phenotype. Replicative senescence can be induced via pathways that accelerate shortening of the telomere, while premature stress-induced senescence can be caused by DNA damage via telomere-independent mechanisms e.g. oxidative stress or UVB exposure (Von Zglinicki et al. 1995; Pascal et al. 2005; Debacq-Chainiaux et al. 2005). The analysis of purified subpopulations of human MF-progenitor fibroblasts derived from skin and obtained

through subcloning procedures indicated that MFI, MFII and MFIII progenitor fibroblasts show increased telomere-shortening along the differentiation sequence MF1-MFII-MFIII (Herskind et al. unpublished results).

As demonstrated for the rabbit kidney two biochemically diverse subpopulations of fibroblasts have been characterized in the cortex and papilla (Rodemann et al. 1991; Knecht et al. 1991). Papillary fibroblasts presented an about 50% longer mitotic lifespan than cortical fibroblasts before both subtypes differentiate into post-mitotic fibrocytes. In addition to differences in protein expression, these two renal fibroblast subtypes respond differentially to growth factor stimulation as well as to stimuli from co-cultured epithelial cells. While no differences in growth response of these two subtypes could be observed after mitogenic stimulation with either epidermal growth factor (EGF) or insulin-like growth factor-1 (IGF-1) a significant differential response could be demonstrated for platelet derived growth factor (PDGF). PDGF exerted a potent mitogenic stimulus for papillary fibroblast but was only weakly mitogenic for cortical fibroblasts (Knecht et al. 1991).

Additional patterns of fibroblasts heterogeneity have been identified through the study of wounding and wound healing. Wound healing proceeds in three inter-related phases (reviewed in Clark et al. 1993): inflammation phase, proliferation phase, and maturation phase. Although fibroblasts have long been recognized as key cells in those process, it is only recently that we have begun to understand the origin and phenotypic differences of fibroblasts as related to the specific phases of wound healing. The initial inflammatory phase consists of an orderly extravasation of leucocytes like PMN and monocytes/macrophages into the wound. Bucala et al. (1994) have shown that a population of fibroblasts which arise from blood-borne circulating progenitor cells migrate into the wound site and may account for as many as 10% of the cells that infiltrate sites of acute tissue injury. These cells were also termed fibrocytes and express markers for cells of both hematopoietic and mesenchymal origin. These fibrocytes have to be clearly distinguished from the fibrocytes described by author and which resemble postmitotic fibroblasts. In addition it is unclear whether Bucala's fibrocyte of hematopoietic origin enters the fibroblast lineage described above. The collagen⁺/vimentin⁺/CD 34⁺/CD 13⁺/CD45⁺ positive fibrocyte cells may contribute to normal wound repair by antigen presentation but may also participate in pathologic fibrotic responses (Chesney et al. 1997). Tredget and his group (Yang et al. 2005) identified this cell type in hypertrophic scarring of the skin.

During the proliferative phase of wound healing granulation tissue and fibrovascular ingrowth occurs. Rossio-Pasquier et al. (1999) have demonstrated that in full thickness dermal loss, fibroblasts migrating into the wound originate from the subcutaneous wound bed rather than from the non injured surrounding dermis. Van den Bogaerd et al. (2002) have shown that mitotic and contractile activity differs significantly between these dermal and fat derived fibroblasts. Middelkoop (2005) suggested that these differences may have a great impact on the use of either acellular dermal substitutes which repopulate from the subcutaneous fat or dermal-derived fibroblast-seeded matrices.

In the final phase of wound healing, a specialized population of differentiated fibroblasts occur (Gabbiani et al. 1971, 2003). It has been demonstrated that fibroblasts under mechanical tension and under the influence of Platelet Derived Growth Factor (PDGF) express stress fibers. This cell type is termed pro-to-myofibroblast. Additional tension, TGF- β and a splice variant of fibronectin promote further differentiation into the myofibroblasts. These contractile myofibroblasts express alpha smooth muscle actin and are involved in the production of extracellular matrix and tissue contraction. Germain et al. (1994) provided evidence that human wound fibroblasts represent a functionally different population than fibroblasts isolated from unwounded dermis. In their analysis they concluded that 30–40% of wound-derived fibroblasts were myofibroblasts whereas only 1% of normal fibroblasts were alpha smooth muscle actin positive. Early studies from Bell et al. (1979) demonstrated that fibroblasts of high population doubling which have left the cell cycle can carry out lattice contraction at least as effectively as cycling cells from early passages, leading the authors to conclude that the contraction of wounds is not due to myofibroblasts. In vivo data indicated that the number of myofibroblasts in the wound is dependent upon the amount of transplanted deeper dermal portion (Rudolph et al. 1991). Unfortunately it remains unclear whether this effect is due to the matrix or the resident cell population. Regardless of whether myofibroblasts are the true motile force for wound contraction, there is general agreement that wound contraction is an active cellular phenomenon depending on the activity of viable fibroblasts. Biological control of these cells either by the matrix or by surrounding cells may minimize scar contraction (Bell 1995; Bell et al. 1981).

Interestingly, and of great importance for the understanding of the biology of chronic wounds, is the fact that a different fibroblast phenotype has been identified in diabetic ulcers when compared to normal undiseased skin as well as healthy skin from non-diabetic volunteers (Loots et al. 1999). Fibroblasts from non-insulin-dependent diabetic ulcers exhibited a lower proliferation capacity and more flattened morphologic appearance (Loots et al. 1999). It is tempting to speculate that this fibroblast population resembles postmitotic fibroblasts. Whether these phenotypic differences in fibroblasts are the underlying cause for non-healing diabetic ulcers or a secondary phenomenon reflecting an altered growth factor profile in this type of wound was not examined. Studies by Morocutti et al. (1996, 1997) revealed that dermal fibroblasts from insulin dependent diabetic patient with progressive fibrosis exhibit a remarkably decreased potential for proliferation as well as increased collagen production correlating with an increased proportion of postmitotic fibrocytes. Interestingly, these altered fibroblasts were not derived from lesional skin but from otherwise healthy skin of these patients. Hasan et al. (1997) also presented evidence that fibroblasts from chronic venous ulcer wounds are phenotypically altered, as a slower proliferation rate of wound derived fibroblasts was noticed. In addition a decreased expression of type 2 TGF- β receptors of ulcer fibroblast was accompanied by a failure to phosphorylate Smad 2, Smad 3, and p42/44 mitogen activating protein kinase (MAPK).

2.3 Functional Effects of Fibroblast Subpopulations

Besides the well established matrix building effect of fibroblasts, there is experimental evidence for a fibroblast-derived mesenchymal–epithelial crosstalk regulating epithelial growth factors (Clark 2003). For example reepithelialisation of cutaneous wounds as a concert of proliferation and migration of keratinocytes is strongly dependent on mesenchymal cell- derived factors.

Fibroblasts are able to secrete IL-6, IL-8, HGF, and KGF all of which are known to stimulate keratinocyte proliferation and migration. Fusenig and his group (Fusenig 1994; Maas-Szabowski et al. 1996) have demonstrated that growth-arrested X-ray-irradiated fibroblasts and proliferating fibroblasts profoundly differ in their profiles of IL-1 alpha stimulated cytokines and growth factor secretion. IL-1 alpha stimulated postmitotic fibroblasts depicted lower transcriptional levels (mRNA) of KGF, IL-1 alpha, IL-8, while HGF mRNA was dramatically increased. However, on the protein level IL-1 alpha-stimulated irradiated fibroblasts presented lower HGF levels than proliferating dermal fibroblasts. In previous work by others (Limat et al. 1989; Waelti et al. 1992) the growth promoting effect of postmitotic fibroblasts on keratinocytes was clearly demonstrated *in vitro*.

As recently published (Nolte et al. 2008), non-stimulated subpopulations of dermal derived MF-progenitor fibroblasts or postmitotic fibrocytes constitutively secrete significantly different amounts of KGF. When compared to progenitor fibroblasts MF II the amount of KGF produced per cell increases significantly in MF III type progenitor cells by a factor of about 2.2 (see also Fig. 2.1). Postmitotic fibrocytes, however, produce more than 3-times more KGF than MFII-type progenitors and app. 1.5-times more than MFIII type progenitor fibroblasts. These observations indicate a specific role of the subtypes of progenitor fibroblasts and postmitotic fibrocytes in the mesenchymal–epithelial interaction and specifically in the homeostasis of the epithelial cell system in skin.

As previously described, TGF- β , another important growth factor is secreted by fibroblasts and counteracts the mitotic effect of KGF on keratinocytes. Among the three isoforms of TGF- β , TGF- β 1 is the most prominent regulator. TGF- β 1 was shown to down regulate epithelial growth and induce differentiation and apoptosis in keratinocytes (Mansbridge and Hanawalt 1988). Hakenjos et al. (2000) and von Pfeil et al. (2002) have previously demonstrated that TGF- β 1 is predominantly produced by MF III type progenitor fibroblasts as well as postmitotic fibrocytes (see also Fig. 2.1). This cytokine plays an important role in the autocrine regulation of the differentiation process of progenitor fibroblasts to functional fibrocytes through the induction of cell cycle inhibitor proteins like p21 and p27 mediating permanent cell cycle arrest in G₀ (Lee and Bae 2002).

The importance of fibroblast/fibrocyte interaction with tissue specific epithelia is also demonstrated by the use of fibroblasts to construct skin substitutes. Fibroblasts seeded into a collagen-GAG matrix were shown to promote rapid epithelial outgrowth on the collagen-GAG matrix as compared to a non seeded collagen-GAG (Boyce et al. 1988, 1995, 2000; Boyce 2001). In contrast, the feeder layer system used to culture keratinocytes utilizes postmitotic fibrocytes, originally derived from

the irradiated transformed mouse 3T3 cell line for co-culture (Rheinwald and Green 1975). There is strong evidence from radiobiological research that irradiation does not necessarily kill the feeder cells, but rather induces terminal differentiation with irreversible growth arrest (Rodemann and Bamberg 1995), yet largely preserves physiologic function in producing growth factors and extracellular matrix proteins (Rodemann and Bamberg 1995; Lara et al. 1996; Hakenjos et al. 2000; von Pfeil et al. 2002). So far however, the functional influence of the secreted cocktail of growth factors and cytokines by the various subpopulations of fibroblasts on keratinocyte proliferation remains unclear. Despite the possible qualitative and quantitative differences between the different subpopulations it is well described that a direct cell-cell contact is crucial to promote keratinocyte growth because fibroblast conditioned medium cannot substitute for feeder cells (Briggaman and Wheeler 1968; Krejci et al. 1991).

In addition to growth promoting experiments, a novel line of research is focusing on site specific interaction of fibroblast subtypes on development and tissue homeostasis. In an elegant set of experiments (Yamaguchi et al. 2005) it was shown that fibroblasts derived from soles and palms are able to induce keratin 9 mRNA in cultured non-palmoplantar keratinocytes. Non-palmoplantar keratinocytes cultured alone or in co-culture with non-palmoplantar fibroblasts failed to express keratin 9, indicating the extrinsic regulation by signals from site specific fibroblasts. In a similar set of experiment site specific regulation of melanocytes and pigmentation was also demonstrated (Yamaguchi et al. 2005).

In the context of functional epithelialization there is evidence that fibroblasts play a major role in basement membrane formation either alone or in conjunction with overlying keratinocytes (Contard et al. 1993; Cooper et al. 1993; Sahuc et al. 1996). These studies have demonstrated that keratinocytes alone were either unable to or limited in the production of laminin 1, collagen IV and laminin 5. In contrast, human fibroblasts, alone or in combination with overlying keratinocytes showed significant production of laminin 1 and collagen IV, and laminin 5. Subsequently, fibroblasts seeded into a collagen-GAG matrix were shown to promote basement membrane formation and epidermal homeostasis as compared to a non seeded collagen-GAG. Total collagen production by fibroblasts is dependent on the subpopulation of fibroblasts with postmitotic fibroblasts producing 5 to 8 times more collagen type I, III or V as compared to progenitor fibroblasts (Rodemann et al. 1989). Moreover, cultured postmitotic fibrocytes in comparison to progenitor fibroblasts produce the in vivo-like proportion of interstitial collagens I, III and V indicating again the important role of this cell type for tissue and ECM homeostasis.

2.4 Functional Effects of Extracellular Matrix on Fibroblasts

While it is well accepted that fibroblasts produce and modulate extracellular matrix there is strong evidence that extracellular matrix material as well as the specific lattice shape regulate cell shape and function of fibroblasts and may act as a form of

switch between proliferative and differentiate states. Eckes et al. (1993) examined fibroblast behaviour in a three-dimensional contracted collagen matrix and compared them to fibroblast monolayers. They found that culture of dermal fibroblasts seeded in collagen gel leads to a time-dependent depression of pro alpha 1(I) collagen mRNA levels. It was not however investigated whether under these conditions fibroblasts changed in morphology or in their receptor expression. There is evidence that activation of the integrin alpha 1 beta 1 leads to down-regulation of collagen synthesis (Langholz et al. 1995). Studies by Nakagawa et al. (1989) on skin fibroblasts demonstrated that collagen embedding influences the cellular response and subsequent growth factor release of some but not all growth factors, e.g. IL-1 β . Contracting lattices placed under tension lead to synthesis of collagen and regulated the expression of cytokines like TGF- beta 1 differently from relaxed collagen lattices embedding (Eckes et al. 2000; Kessler et al. 2001). Unfortunately phenotypic changes of these embedded fibroblasts were not investigated.

Different matrix materials were also shown to have a significant influence on fibroblast function. Normal human cultured fibroblasts undergo programmed cell death in a three-dimensional contractile collagen gel while fibrin gels did not exert this effect (Fluck et al. 1998). In addition fibroblasts did contract the fibrin gel but did not proliferate. In anchored collagen gels fibroblasts showed a doubling time of 2 days and no apoptotic cell death. Despite all this evidence only limited in vivo studies have been undertaken to objectively evaluate scarring as a function of different dermal matrices, which is of crucial interest for dermal engineering.

Mansbridge and his group (Kern et al. 2001, 2002) analysed fibroblasts behaviour in monolayer collagen cultures and scaffold based three-dimensional cultures with special attention to the expression of molecules associated with immune system activation. In monolayers of dermal fibroblasts expression of HLA class I but not of HLA-DR was found. Induction of HLA-DR and an increase in HLA -class I expression was observed after interferon-gamma stimulation. In contrast fibroblasts cultured in three-dimensional scaffolds consisted of a fraction of fibroblasts which were non-responsive to gamma interferon. After isolation and subsequent monolayer culture this effect was reversible. Interestingly, collagen-gel-based three-dimensional fibroblast cultures did not exert a similar unresponsiveness and it was concluded that the interaction of fibroblasts with naturally deposited extracellular matrix other than collagen was responsible for this effect. Kern et al. (2002) examined the expression of CD40 on fibroblasts as the interaction of fibroblasts and T-lymphocytes through the CD40 receptor was suggested to contribute to fibrogenesis. Human dermal fibroblasts express CD40 in a manner that is inversely related to proliferation and it was concluded that CD40 expression is greater in the stationary phase than in the log phase. In three-dimensional scaffold based fibroblast cultures responsiveness to interferon-gamma with subsequent CD40 expression was significantly reduced providing a possible etiology for the lack of rejection of allergenic three-dimensional scaffold based fibroblast cultures. This effect appears to be significantly dependent upon the scaffold utilized as Navsaria and his group (Griffiths et al. 2004) and Lamme et al. (2002) have shown that allergenic fibroblasts seeded in a collagen matrix do not persist but rather induce a fibrotic response.

2.5 Concluding Remarks

As reviewed in all investigated tissue and organ systems fibroblasts represent a functional diverse population of cells. Phenotypic differences are manifested in a variety of ways: extracellular matrix production and organization as well as the secretion of and response to growth factors. The functional diversity of the different fibroblast subtypes have to be considered when aspects of tissue homeostasis, especially with respect to stromal or mesenchymal-epithelial interactions and cross-talk are discussed. For example as stated by Sorrel and Caplan (2004) for dermal fibroblasts the term “fibroblast” is an oversimplification. All investigations into phenotypic characteristics of stromal fibroblasts indicated that these cells represent a dynamic and diverse population of functional cell types. A specific nomenclature should reflect this fact and greater care should be taken when defining the population of fibroblasts used in experimental studies. The terminology fibrocyte should be reserved for the postmitotic subtype of fibroblasts as the term ‘cyte’ implicates a non-proliferative cell population.

References

- Baglolle CJ, Ray DM, Bernstein SH, Feldon SE, Smith TJ (2006) More than structural cells, fibroblasts create and orchestrate the tumor microenvironment. *Immunol Invest* 35:297–325
- Bayreuther K, Rodemann HP, Francz PI, Maier K (1988) Differentiation of fibroblast stem cells. *J Cell Sci Suppl* 10:115–130
- Bayreuther K, Rodemann HP, Hommel R, Dittmann K, Albiez M, Francz PI (1989) Human skin fibroblasts in vitro differentiate along a terminal cell lineage. *Proc Natl Acad Sci U S A* 85:5112–5116
- Beacham DA, Cukierman E (2005) Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. *Semin Cancer Biol* 15:329–341
- Bell E (1995) Strategy of the selection of scaffolds for tissue engineering. *Tissue Eng* 1:163–179
- Bell E, Ivarsson B, Merrill C (1979) Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc Natl Acad Sci U S A* 76:1274–1278
- Bell E, Ehrlich HP, Sher S, Merrill C, Sarber R, Hull B, Nakatsuji T, Church D, Buttle DJ (1981) Development and use of a living skin equivalent. *Plast Reconstr Surg* 67:386–392
- Boyce ST (2001) Design principles for the composition and performance of cultured skin substitutes. *Burns* 27:523–533
- Boyce ST, Christianson D, Hansbrough JF (1988) Structure of a collagen-GAG skin substitute optimized for cultured human epidermal keratinocytes. *J Biomed Mater Res* 22:939–957
- Boyce ST, Goretsky MJ, Greenhalgh DG, Kagan RJ, Rieman MT, Warden GD (1995) Comparative assessment of cultured skin substitutes and native skin autograft for the treatment of full thickness burns. *Ann Surg* 222:743–752
- Boyce ST, Supp AT, Wickett RR, Hoath SB, Warden GD (2000) Assessment with the dermal torqueometer of skin pliability after treatment of burns with cultured skin substitutes. *J Burn Care Rehabil* 21:55–63
- Briggaman RA, Wheeler CE (1968) Epidermal-dermal interactions in adult human skin: role of dermis in epidermal maintenance. *J Invest Dermatol* 51:454–465
- Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A (1994) Circulating fibrocytes define a new leukocyte subpopulations that mediates tissue repair. *Mol Med* 1:71–81

- Burger A, Löffler H, Bamberg M, Rodemann HP (1998) Molecular and cellular basis of radiation fibrosis. *Int J Radiat Biol* 73:401–408
- Castor CW, Prince RK, Dorstewitz EL (1962) Characteristics of human “fibroblasts” cultivated in vitro from different anatomical sites. *Lab Invest* 11:703–713
- Chang HY, Chi JT, Dudoit S, Bondre C, Van de Rijn M, Botstein D, Brown PO (2002) Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci* 99:12877–12882
- Chesney J, Bacher M, Bender A, Bucala R (1997) The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. *Proc Natl Acad Sci U S A* 94:6307–6312
- Chipev CC, Simon M (2002) Phenotypic differences between dermal fibroblasts from different body sites determine their responses to tension and TGFbeta1. *BMC Dermatol* 2:13
- Clark RAF (2003) Epithelial-mesenchymal networks in wounds: a hierarchical view. *Commentary. J Invest Dermatol* 120:9–11
- Clark RAF, Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (eds) (1993) Mechanisms of cutaneous wound repair; dermatology in general medicine. McGraw Hill, New York, pp 473–486
- Contard P, Bartel RL, Jacobs L, Perlish JS, MacDonald ED, Handler L, Cone D, Fleischmajer R (1993) Culturing keratinocytes and fibroblasts in a three-dimensional mesh results in epidermal differentiation and formation of a basal lamina-anchoring zone. *J Invest Dermatol* 100:35–59
- Cooper ML, Andree C, Hansbrough JF, Zapata-Sirvent RL, Spielvogel RI (1993) Direct comparison of a cultured composite skin substitute containing human keratinocytes and fibroblasts to an epidermal sheet graft containing human keratinocytes on athymic mice. *J Invest Dermatol* 101:811–819
- Debacq-Chainliaux F, Borlon C, Pascal T, Royer V, Eliaers F, Ninane N, Carrard G, Friguet B, de Longueville F, Boffe S, Remacle J, Toussaint O (2005) Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. *J Cell Sci* 118:743–758
- Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubel JJ, Pereira-Smith O, Peacocke M, Campisi J (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 92:9363–9367
- Eckes B, Mauch C, Hüppe G, Krieg T (1993) Downregulation of collagen synthesis in fibroblasts within three-dimensional collagen lattices involves transcriptional and posttranscriptional mechanisms. *FEBS* 216:129–133
- Eckes B, Kessler D, Aumailley M, Krieg T (2000) Interactions of fibroblasts with the extracellular matrix: implications for the understanding of fibrosis. *Springer Semin Immunopathol* 21:415–429
- Fluck J, Querfeld C, Cremer A, Niland S, Krieg T, Sollberg S (1998) Normal human primary fibroblasts undergo apoptosis in three-dimensional contractile collagen gels. *J Invest Dermatol* 110:153–157
- Fusenig NE (1994) Epithelia-mesenchymal interactions regulate keratinocyte growth and differentiation in vitro. In: Leigh I, Lane B, Watt F (eds) *The keratinocyte handbook*. Cambridge University Press, Cambridge, pp 71–94
- Gabbiani G (2003) The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol* 200:500–503
- Gabbiani G, Ryan GB, Majno G (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* 27:549–550
- Germain L, Jean A, Auger F, Garrel DR (1994) Human wound healing fibroblasts have greater contractile properties than dermal fibroblasts. *J Surg Res* 57:268–273
- Griffiths M, Ojeh N, Livingstone R, Price R, Navsaria H (2004). Survival of Apligraf in acute human wounds. *Tissue Eng* 10:1180–1195
- Gron B, Stoltze K, Andersson A, Dabelsteen E (2002) Oral fibroblasts produce more HGF and KGF than skin fibroblasts in response to co-culture with keratinocytes. *APMIS* 110:892–898

- Hakenjos L, Bamberg M, Rodemann HP (2000) TGF- β 1-mediated alterations of rat lung fibroblast differentiation resulting in the radiation-induced fibrotic response. *Int J Radiat Biol* 76:503–509
- Hasan A, Murata H, Falabella A, Ochoa S, Zhou L, Badiavas E, Falanga V (1997) Dermal fibroblasts from venous ulcers are unresponsive to the action of transforming growth factor-beta1. *J Dermatol Sci* 16:59–66
- Herskind C, Bentzen SM, Overgaard J, Bamberg M, Rodemann HP (1998) Differentiation state of skin fibroblast cultures versus risk of subcutaneous fibrosis after radiotherapy. *Radiother Oncol* 47:263–269
- Kern A, Liu K, Mansbridge J (2001) Modification of fibroblast gamma-interferon responses by extracellular matrix. *J Invest Dermatol* 117:112–118
- Kern A, Liu K, Mansbridge J (2002) Modulation of interferon-gamma response by dermal fibroblast extracellular matrix. *Ann N Y Acad Sci* 961:364–367
- Kessler D, Dethlefsen S, Haase I, Plomann M, Hirche F, Krieg T, Eckes B (2001) Fibroblasts in mechanically stressed collagen lattices assume a “synthetic” phenotype. *J Biol Chem* 276:36575–36585
- Knecht A, Fine LG, Kleinman KS, Rodemann HP, Müller GA, Woo DD, Norman JT (1991) Fibroblasts of rabbit kidney in culture. II. Paracrine stimulation of papillary fibroblasts by PDGF. *Am J Physiol* 261:F292–F299
- Krejci NC, Cuono CB, Langdon RC, McGuire J (1991) In vitro reconstitution of skin: fibroblasts facilitate keratinocyte growth and differentiation on acellular reticular dermis. *J Invest Dermatol* 97:843–848
- Lamme EN, van Leeuwen RTJ, Brandsma K, van Marle J, Middelkoop E (2000) Higher number of autologous fibroblasts in an artificial dermal substitute improve tissue regeneration and modulate scar-tissue formation. *J Pathol* 190:595–603
- Lamme EN, van Leeuwen RT, Mekkes JR, Middelkoop E (2002) Allogeneic fibroblasts in dermal substitutes induce inflammation and scar formation. *Wound Repair Regen* 10:152–160
- Langholz O, Rockel D, Mauch C, Kozłowska E, Bank I, Krieg T, Eckes B (1995) Collagen and collagenase expression in three-dimensional collagen lattices are differentially regulated by alpha 1 beta 1 and alpha 2 beta 1 integrins. *J Cell Biol* 131:1903–1915
- Lara PC, Russell NS, Smolders IJ, Bartelink H, Begg AC, Coco-Martin JM (1996) Radiation-induced differentiation of human skin fibroblasts: relationship with cell survival and collagen production. *Int J Radiat Biol* 70:683–692
- Lee KY, Bae SC (2002) TGF-beta-dependent cell growth arrest and apoptosis. *J Biochem Mol Biol* 35:47–53
- Limat A, Hunziker T, Boillat C, Bayreuther K, Noser F (1989) Postmitotic human dermal fibroblasts efficiently support the growth of human follicular keratinocytes. *J Invest Dermatol* 92:758–762
- Liotta LA, Kohn EC (2001) The microenvironment of the tumor-host interface. *Nature* 411:375–379
- Loots MA, Lamme EN, Mekkes JR, Bos JD, Middelkoop E (1999) Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. *Arch Dermatol Res* 291:93–99
- Maas-Szabowski N, Fusenig NE (1996) Interleukin-1-induced growth factor expression in postmitotic and resting fibroblasts. *J Invest Dermatol* 107:849–855
- Mansbridge JN, Hanawalt PC (1988) Role of transforming growth factor beta in the maturation of human epidermal keratinocytes. *J Invest Dermatol* 90:336–341
- Micke P, Ostman A (2005) Exploring the tumor environment: cancer associated fibroblasts as targets in cancer therapy. *Expert Opin Ther Targets* 9:1217–1233
- Middelkoop E (2005) Fibroblast phenotypes and their relevance for wound healing. *Int J Low Extrem Wounds* 4:9–11
- Mollenhauer J, Bayreuther K (1986) Donor-age-related changes in the morphology, growth potential, and collagen biosynthesis in rat fibroblast subpopulations in vitro. *Differentiation* 32:165–172

- Morocutti A, Earle KA, Sethi M, Piras G, Pal K, Richards D, Rodemann HP, Viberti GC (1996) Premature senescence of skin fibroblasts from insulin-dependent diabetic patients with kidney disease. *Kidney Int* 50:250–256
- Morocutti A, Earle KA, Rodemann HP, Viberti GC (1997) Premature cell ageing and evolution of diabetic nephropathy. *Diabetologia* 40:244–246
- Nakagawa S, Pawelek P, Grinnell F (1989) Long-term culture of fibroblasts in contracted collagen gels: effects on cell growth and biosynthetic activity. *J Invest Dermatol* 93:792–798
- Nolte S, Xu W, Rennekampff H-O, Rodemann HP (2008) Diversity of fibroblasts—a review on implications for skin tissue engineering. *Cell Tiss Org* 187:165–176
- Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD (1999) Carcinoma-associated fibroblasts direct tumor progression of initiated human prostate epithelium. *Cancer Res* 59:5002–5011
- Orimo A, Gupta PB, Segroi DC, Renzana-Seisdedos F, Delaunay T (2005) Stromal fibroblasts present in invasive human breast carcinomas promote growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 121:335–348
- Pascal T, Debacq-Chainliaux F, Chrétien A, Bastin C, Dabée AF, Bertholet V, Remacle J, Toussaint O (2005) Comparison of replicative senescence and stress-induced premature senescence combining differential display and low-density DNA arrays. *FEBS Lett* 579:3651–3659
- Proia DA, Kuperwasser C (2005) Stroma: tumor agonist or antagonist. *Cell Cycle* 4:1022–1025
- Rheinwald JG, Green H (1975) Feeder layer system: serial cultivation of strains of human epidermal keratinocytes. *Cell* 6:331–343
- Rinn JL, Bondre C, Gladstone HB, Brown PO, Chang HY (2006) Anatomic demarcation by positional variation in fibroblast gene expression programs. *PLoS Genet* 2:1084–1096
- Rinn JL, Wang JK, Liu H, Montgomery K, van de Rijn M, Chang HY (2008) A systems biology approach to anatomic diversity of skin. *J Invest Dermatol* 128:776–782
- Rodemann HP (1989) Differential degradation of intracellular proteins in human skin fibroblasts of mitotic and mitomycin C(MMC)-induced postmitotic differentiation states. *Differentiation* 42:37–43
- Rodemann HP (1993) Differential gene expression, protein synthesis and degradation in ageing fibroblasts. In: Bernd A, Bereiter-Hahn J, Hevert F, Holzmann H (eds) *Cell culture models for dermatological research*. Springer, Berlin, pp 272–277
- Rodemann HP, Bamberg M (1995) Cellular basis of radiation-induced fibrosis. *Radiother Oncol* 35:83–90
- Rodemann HP, Mueller GA (1990) Abnormal growth, clonal proliferation and 35S-methionine polypeptide pattern of fibroblasts derived from kidneys with interstitial fibrosis. *Proc Soc Exp Biol Med* 195:57–63
- Rodemann HP, Bayreuther K, Francz PI, Dittmann K, Albiez M (1989) Selective enrichment and biochemical characterisation of seven fibroblast cell types of human skin fibroblast populations in vitro. *Exp Cell Res* 180:84–93
- Rodemann HP, Müller GA, Knecht A, Norman JT, Fine LG (1991) Fibroblasts of rabbit kidney in culture: I. characterization and identification of cell-specific markers. *Am J Physiol* 261:283–291
- Rodemann HP, Binder A, Burger A, Löffler H, Bamberg M (1996) The underlying cellular mechanisms of fibrosis. *Kidney Int* 49:32–36
- Rossio-Pasquier P, Casanova D, Jomard A, Dermarchez M (1999) Wound healing of human skin transplanted onto the nude mouse after a superficial excisional injury: human dermal reconstruction is achieved in several steps by two different fibroblast subpopulations. *Arch Dermatol Res* 291:591–599
- Rudolph R, Vande J, Berg G, Pierce F (1991) Changing concept in myofibroblast function and control. In: Janssen H, Rooman JIS (eds) *Wound healing*. Wrightson Biomedical Publishing Ltd., Petersfield, pp 103–115

- Sahuc F, Nakazawa K, Berthod F, Collombel C, Damour O (1996) Mesenchymal-epithelial interactions regulate gene expression of type VII collagen and kalinin in keratinocytes and dermal-epidermal junction formation in a skin equivalent model. *Wound Rep Regen* 4:93–102
- Sorrell JM, Caplan AI (2004) Fibroblast heterogeneity: more than skin deep. *J Cell Sci* 117:667–675
- Stephens P, Davies KJ, Occeleston N, Pleass RD, Kon C, Daniels J, Khaw PT, Thomas DW (2001) Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix organization and matrix metalloproteinase activity. *Br J Dermatol* 144:229–237
- Stuelten CH, Busch JI, Tang B, Flanders KC, Oshima A, Sutton E, Karpova TS, Roberts AB, Wakefield LM, Niederhuber JE (2010) Transient tumor-fibroblast interactions increase tumor cell malignancy by TGF- β mediated mechanism in a mouse xenograft model of breast cancer. *PlosOne* 5:e9832
- Van Den Bogaerd AJ, van Zuijlen PPM, van Galen M, Lamme EN, Middelkoop E (2002) The suitability of cells from different tissues to be used in tissue engineered skin substitutes. *Arch Dermatol Res* 294:135–142
- von Pfeil A, Hakenjos L, Herskind C, Dittmann K, Weller M, Rodemann HP (2002) Irradiated homozygous TGF-1 knockout fibroblasts show enhanced clonogenic survival as compared with TGF-1 wild-type fibroblasts. *Int J Radiat Biol* 78:331–339
- Von Zglinicki T, Saretzki G, Docke W, Lotze C (1995) Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res* 220:186–193
- Waelti ER, Inaebnit SP, Rast HP, Hunziker T, Limat A, Braathen LR, Wiesmann U (1992) Co-culture of human keratinocytes on post-mitotic human dermal fibroblast feeder cells: production of large amounts of interleukin-6. *J Invest Dermatol* 98:805–808
- Yamaguchi Y, Hearing VJ, Itami S, Yoshikawa K, Katayama I (2005) Mesenchymal-epithelial interactions in the skin: aiming for site specific tissue regeneration. *J Dermatol Sci* 40:1–9
- Yang L, Scott PG, Dodd C, Medina A, Jiao H, Shankowsky HA, Ghahary A, Tredget EE (2005) Identification of fibrocytes in postburn hypertrophic scars. *Wound Repair Regen* 13:398–404

Tumor-Associated Fibroblasts and their Matrix
(Eds.) M.M. Mueller; N.E. Fusenig
2011, XV, 452 p. 73 illus., 52 in color., Hardcover
ISBN: 978-94-007-0658-3

iverio®

lib