

Fibroblast phenotype plasticity: relevance for understanding heterogeneity in "fibroblastic" tumors

Brian Eyden PhD, Department of Histopathology, Christie Hospital NHS Trust,
Manchester M20 4BX, UNITED KINGDOM
Tel +44 161 446 3292 Fax +44 161 446 3300
Email brian.eyden@christie-tr.nwest.nhs.uk

Introduction

In the tumors or tumor-like lesions which we instinctively regard as fibroblastic, there is, as in other tumor groups, a wide range of cellular differentiation, which we can explain or rationalise in terms of phenotypic plasticity of the "normal" fibroblast. In this process, differentiation can be altered by molecules of external origin, which interact with surface receptors, and induce cascades of molecular interactions: these eventually activate gene expression, lead to translation of mRNA into proteins, organization of proteins into supramolecular complexes (organelles), and hence ultimately to different cellular appearances, i.e., phenotypes.

In this paper, the various kinds of fibroblast transformation are discussed, and some insights provided into the molecular mechanisms driving these transdifferentiation processes. Clearly, comparable molecular events may be taking place in abnormal or neoplastic fibroblasts to produce the heterogeneous tumors, which we nevertheless identify as fibroblastic. The objective of this paper, therefore, is to provide a basis for understanding the diverse phenotypes expressed by fibroblastic tumors or lesions. The most studied transformation - that of the fibroblast to the myofibroblast - will be emphasized, although other examples of transdifferentiation of relevance to fibroblastic tumors will be mentioned.

In comparing the differentiation of fibroblasts vis-à-vis their neoplastic counterparts, the following broad categories come to mind:

- "pure" fibroblastic differentiation → "pure" fibroblastic tumors
- myofibroblastic transdifferentiation → myofibroblastic tumors
- fibroblasts transforming into histiocytes → fibrohistiocytic tumors
- fibroblasts undergoing adipocytic differentiation → lipogenic tumors.

Lipogenic and fibrohistiocytic differentiation in relation to fibroblasts

Definition of the fibroblast Fibroblasts are spindled cells with a cytoplasm dominated by rough endoplasmic reticulum (rER). In addition, they have subplasmalemmal densities [1,2], a big Golgi apparatus, and sometimes collagen secretion granules are seen [3], but no myofilaments or lamina.

Fibroblasts, lipogenesis and liposarcoma A number of studies have suggested that the fibroblast is a stem cell for adipocytes [4-6]. This fibroblast itself may derive from a less differentiated mesenchymal cell. In white adipose tissue, Napolitano [4] has shown initial lipid synthesis in spindled fibroblastic cells, which then increase their lipogenic activity, develop a more rounded cell morphology and elaborate a lamina. Such an origin for the adipocyte would explain why some spindle-cell lipogenic tumors, such as spindle-cell liposarcoma, retain some morphological reminiscences of a fibroblastic precursor stem cell and may even resemble fibrosarcoma.

Clues to the molecular mechanisms by which fibroblasts switch to lipogenic activity in the normal and neoplastic state are beginning to emerge. For example, it has been suggested that human fibroblast subsets exist, defined by presence or absence of the thy-1 receptor. Those possessing the receptor are directed towards a myofibroblastic phenotype (discussed below), while thy-1-negative fibroblasts exclusively develop a lipofibroblastic phenotype [7]. Liposarcoma has been

shown to be initiated by a specific protein domain within a fusion gene product (CHOP) resulting from the most common chromosomal translocation in myxoid liposarcoma, t(12;16)(q13;p11) [8]. CHOP has also been found to prevent adipocyte differentiation [9], an observation which goes at least some way to explaining why so many of the more spindled cells in myxoid liposarcoma show minimal lipid synthesis.

The fibroblast-histiocyte relationship The overlapping features of monocytes or macrophages, on the one hand, and fibroblasts, on the other [10], and the possibility that they could transdifferentiate one to another [11, 12] has been recognized for some time. Examples include:

- Endometrial fibroblasts producing collagen in the proliferative phase are the same cells which then phagocytose collagen in the premenstrual phase [13, 14].
- The pericryptal fibroblast of the colonic mucosa develops morphological and histochemical features of macrophages as it migrates to subtend the free surface epithelium [14, 15].
- Corneal stromal fibroblasts behave as macrophages when presented with colloidal material and synthesise acid hydrolases for intracellular digestion [14, 16].
- Skin fibroblasts can be converted to tissue histiocytes by Snyder-Theilen feline sarcoma virus [17, 18].
- Blood monocytes have been reported to transform into fibroblasts *in vitro* [19-21].
- Macrophages have been observed to transdifferentiate into fibroblasts as a result of Schistosoma mansoni infection [22, 23].

These examples of fibroblasts and macrophages interconverting into one another are reflected in the day-to-day experience of ultrastructurally orientated pathologists who often see at least modest levels of phagocytic activity in a wide variety of fibroblastic cells and lesions. These range from ingested melanin in dermal fibroblasts to the co-expression of lysosomes and rER in the fibrohistiocytic (or histiofibroblastic [24]) cells of such tumors as malignant fibrous histiocytoma. The basic mechanism by which a fibroblast or primitive mesenchymal cell might differentiate towards a macrophage is far from understood. However, we know of a number of cell surface and other proteins which characterize macrophages - carboxypeptidase M [25] and the protein product of the CHI3L1 gene [26], for example - and we know of an increasing number of molecules which appear to promote macrophage differentiation from precursor cells, such as 14-membered macrolide compounds [27] and Imatinib [28]. These data in normal cells could form the basis for understanding the development of fibrohistiocytic differentiation in tumors.

Fibroblast-myofibroblast transformation

Definition of the myofibroblast The main features of the myofibroblast include vimentin and α -smooth-muscle actin (α SMA) immunostaining (as well as desmin in certain lesional myofibroblasts), and an ultrastructure based largely on prominent rough endoplasmic reticulum, sparse peripheral bundles of myofilaments with focal densities, and fibronexus junctions [29-31] but not lamina.

Induction of the myofibroblast phenotype by TGF β One of the principal differences between fibroblasts and myofibroblasts is the absence of α SMA and myofilaments in fibroblasts and their presence in the myofibroblast. Results from a variety of sources suggest that the primary mechanism for the *de novo* synthesis of α SMA requires the combined action of growth factors, principally transforming growth factor- β (TGF β) and platelet-derived growth factor, matrix molecules such as cellular fibronectin, and mechanical stress [32, 33]. TGF β can be produced by malignant cells, and can target receptors on tumor stromal fibroblasts, which then transdifferentiate into early myofibroblasts by virtue of α SMA synthesis [32, 33]. Studies of TGF β in fibroblastic and myofibroblastic lesions are so far limited [34], and it remains to be seen from future research whether TGF β , as might be expected, is present in developing

myofibroblastic lesions, and minimally expressed or absent in purely fibroblastic tumors such as giant-cell fibroblastoma.

Since all biological systems show variation and a spectrum of appearances, we cannot expect there to be a rigid distinction between fibroblastic and myofibroblastic lesions. This is illustrated by fibroblasts grown *in vitro*, in basal cell carcinoma stroma, and in adult fibrosarcoma tumor cells. Basal cell carcinoma possesses a stroma, which, although exhibiting α SMA-staining [35], contains less of the fully differentiated myofibroblasts typically seen in squamous cell carcinoma (Brian Eyden, unpublished observation). Instead, one may see cells, which are all but classical fibroblasts, with abundant rER, subplasmalemmal linear densities, a big Golgi apparatus and collagen secretion granules. Closer scrutiny of some of these cells, though, reveals very modestly developed bundles of subplasmalemmal actin filaments with small numbers of focal densities. Although these cells are predominantly fibroblasts, they are nevertheless showing the very earliest signs of actin synthesis and myofilament elaboration - the earliest indications, in short, of myofibroblastic differentiation. On the basis of our knowledge of the effects of TGF β we might perhaps expect this growth factor to be instrumental in the conversion of stromal fibroblasts to these very early "myofibroblasts", but also perhaps TGF β to be much more upregulated in the stroma of squamous cell carcinoma, where numerous fibronexus-bearing myofibroblasts are present [30, 31].

Similarly, in fibrosarcoma, the classical herringbone pattern seen in histological sections and the poor staining for α SMA, might predict a purely fibroblastic ultrastructure. Fibrosarcoma does, as expected, have abundant rER, but frequently also there are modest numbers of peripheral myofilaments [36, 37]: even a primitive attempt at fibronexus-formation has been noted [31]. In such tumors, one might expect low levels of TGF β indicating a low level of activation of the myofibroblastic phenotype. Future work is needed to confirm these ideas.

The development of α SMA or myofilaments as seen in the myofibroblast is a widespread subcellular reaction in pathology being found in a very wide range of normal, reactive and neoplastic cells *in vitro* and *in vivo* [38]. This *de novo* synthesis of α SMA or myofilaments appears to be a common expression of a pathological state, produced by external trauma (such as wounding *in vivo* or cell cultivation *in vitro*) or inherent abnormality such as malignant transformation. This widespread distribution could be explained by the equally widespread presence of TGF β . It provides something of an explanation as to why we so often see modestly developed myofilaments or low levels of α SMA in the course of our routine diagnostic work, including, for example, otherwise unambiguously non-smooth-muscle tumors such as chondromyxoid fibroma [39-41], chondroblastoma [42], osteosarcoma [43, 44] and rhabdomyosarcoma [45].

Summary

This paper describes some of the principal forms of differentiation exhibited by "pure" and transforming fibroblasts as a means of explaining the phenotypic variation found in fibroblastic tumors. Some of the molecular mechanisms driving the differentiation processes are discussed. While we are beginning to identify some of the characteristic molecules on the surfaces of fibroblasts and their transformed variants, we are far from understanding a number of features which bear on the subject of the diverse phenotypes encountered in fibroblastic tumors. We know hardly anything of the mechanism by which cellular growth patterns are generated, such as the herringbone or the storiform growth pattern in fibrosarcoma and fibrohistiocytic tumors respectively; or, of the mechanism directing and controlling nuclear shape, which might explain why a cell produces a spindled nucleus characteristic of a fibroblast or a reniform nucleus typical of a macrophage, still less the characteristic longitudinal nuclear fissures of, for example, Dermatofibrosarcoma protuberans, all of which features are important to the tumor pathologist. Investigations into the mechanisms of differentiation in normal fibroblasts could prove fertile ground for defining comparable differentiation in tumors.

References

1. Kawanami O, Ferrans VJ, Crystal RG. Subplasmalemmal linear densities in cells of the mononuclear phagocyte system in lung. *Am J Pathol.* 1980; 100: 131-150.
2. Mirra SS, Miles ML. Subplasmalemmal linear density. A mesodermal feature and a diagnostic aid. *Hum Pathol.* 1982; 13: 365-380.
3. Eyden BP. Collagen secretion granules in reactive stromal myofibroblasts, with preliminary observations on their occurrence in spindle cell tumours. *Virchows Arch [A].* 1989; 415: 437-445.
4. Napolitano L. The differentiation of white adipose cells. An electron microscope study. *J Cell Biol.* 1963; 18: 663-679.
5. Wasserman F. The development of adipose tissue. In: Renold AE and Cahill GF eds. *Handbook of physiology: adipose tissue.* Baltimore: Williams and Wilkins; 1965: 87-90.
6. Slavin BG. Fine structural studies on white adipocyte differentiation. *Anat Rec.* 1979; 195: 63-72.
7. Koumas L, Smith TJ, Feldon S, Blumberg N, Phipps RP. Thy-1 expression in human fibroblast subsets defines myofibroblastic or lipofibroblastic phenotypes. *Am J Pathol.* 2003; 163: 291-300.
8. Perez-Losada J, Sanchez-Martin M, Rodriguez-Garcia MA, Perez-Mancera PA, Pintado B, Flores T, Battaner E, Sanchez-Garcia I. Liposarcoma initiated by FUS/TLS-CHOP: the FUS/TLS domain plays a critical role in the pathogenesis of liposarcoma. *Oncogene.* 2000; 19: 6015-6022.
9. Adelmant G, Gilbert JD, Freytag SO. Human translocation liposarcoma-CCAAT/enhancer binding protein (C/EBP) homologous protein (TLS-CHOP) oncoprotein prevents adipocyte differentiation by directly interfering with C/EBPbeta function. *J Biol Chem.* 1998; 273:15574-15581.
10. Marchisio PC, Cirillo D, Teti A, Zambonin-Zallone A, Tarone G. Rous sarcoma virus-transformed fibroblasts and cells of monocytic origin display a peculiar dot-like organization of cytoskeletal proteins involved in microfilament-membrane interactions. *Exp Cell Res.* 1987; 169: 202-214.
11. Kouri J, Ancheta O. Transformation of macrophages into fibroblasts. *Exp Cell Res.* 1972; 71; 168-176.
12. Feigl W, Susani M, Ulrich W, Matejka M, Losert U, Sinzinger H. Organisation of experimental thrombosis by blood cells. Evidence of the transformation of mononuclear cells into myofibroblasts and endothelial cells. *Virchows Arch A Pathol Anat Histopathol.* 1985; 406:133-148.
13. Wiencke EC, Cavazos F, Hall DG, Lucas FV. Ultrastructure of human endometrial stromal cell during menstrual cycle. *Am J Obstet Gynecol.* 1968; 102: 65-77.
14. Kaye GI. The futility of electron microscopy in determining the origin of poorly differentiated soft tissue tumors. *Prog Surg Pathol.* 1981; 3: 171-179.
15. Kaye GI, Lane N, Pascal RR. The colonic pericryptal fibroblast sheath: replication, migration and cytodifferentiation of a mesenchymal cell system in adult tissue. *Gastroenterology.* 1968; 54: 852-865.
16. Kaye GI, Pappas GD. Studies on the cornea.I. The fine structure of the rabbit cornea and the uptake and transport of colloidal particles by the cornea *in vivo.* *J Cell Biol.* 1962; 12: 457-479.
17. Kopelovich L, Wang TY. Histiocytic conversion of human adult skin fibroblasts by the Snyder-Theilen feline sarcoma virus. *Eur Cytokine Netw.* 1990; 1: 157-168.
18. Kopelovich L. Conversion of human fibroblasts to tissue macrophages by the Snyder-Theilen feline sarcoma virus (ST:FeSV) is associated with the de-novo expression of IL-1 alpha, IL-1 beta, IFN-alpha, TNF-alpha, GM-CSF, and CD4. *Eur Cytokine Netw.* 1991; 2: 99-106.
19. Labat ML, Binguier AF, Seebold C, Moricard Y, Meyer-Mula C, Laporte P, Talmage RV, Grubb SA, Simmons DJ, Milhaud G. Monocytic origin of fibroblasts: spontaneous transformation of blood monocytes into neo-fibroblastic structures in osteomyelosclerosis and Engelmann's disease. *Biomed Pharmacother.* 1991; 45:289-299.

20. Bringuier AF, Seebold-Choqueux C, Moricard Y, Simmons DJ, Milhaud G, Labat ML. T-lymphocyte control of HLA-DR blood monocyte differentiation into neo-fibroblasts. Further evidence of pluripotential secreting functions of HLA-DR monocytes, involving not only collagen but also uromodulin, amyloid-beta peptide, alpha-fetoprotein and carcinoembryonic antigen. *Biomed Pharmacother.* 1992; 46: 91-108.
21. Labat ML, Bringuier AF, Arys-Philippart C, Arys A, Wellens F. Monocytic origin of fibrosis. In vitro transformation of HLA-DR monocytes into neo-fibroblasts: inhibitory effect of all-trans retinoic acid on this process. *Biomed Pharmacother.* 1994; 48:103-111.
22. Godoy M, Geuskens M, Van Marck EA, Borojevic R, Van Gansen P. Schistosomiasis and in vitro transdifferentiation of murine peritoneal macrophages into fibroblastic cells. *Parasitol Res.* 1989; 76:150-161.
23. Bertrand S, Godoy M, Semal P, Van Gansen P. Transdifferentiation of macrophages into fibroblasts as a result of *Schistosoma mansoni* infection. *Int J Dev Biol.* 1992; 36:179-184.
24. Antonescu CR, Erlandson RA, Huvos AG. Primary fibrosarcoma and malignant fibrous histiocytoma of bone - a comparative ultrastructural study: evidence of a spectrum of fibroblastic differentiation. *Ultrastruct Pathol.* 2000; 24: 83-91.
25. Gottfried E, Faust S, Fritsche J, Kunz-Schughart LA, Andreesen R, Miyake K, Kreutz M. Identification of genes expressed in tumor-associated macrophages. *Immunobiology.* 2003;207:351-359.
26. Rehli M, Niller HH, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, Krause SW. Transcriptional Regulation of CHI3L1, a Marker Gene for Late Stages of Macrophage Differentiation. *J Biol Chem.* 2003; 278: 44058-44067.
27. Sunazuka T, Yoshida K, Oohori M, Otaguro K, Harigaya Y, Iwai Y, Akagawa KS, Omura S. Effect of 14-membered macrolide compounds on monocyte to macrophage differentiation. *J Antibiot (Tokyo).* 2003; 56:721-724.
28. Dewar AL, Domaschewitz RM, Doherty KV, Hughes TP, Lyons AB. Imatinib inhibits the in vitro development of the monocyte/macrophage lineage from normal human bone marrow progenitors. *Leukemia.* 2003;17:1713-1721.
29. Eyden BP. Brief review of the fibronexus and its significance for myofibroblastic differentiation and tumor diagnosis. *Ultrastruct Pathol.* 1993; 17: 611-622.
30. Eyden B: The myofibroblast: an assessment of controversial issues and a definition useful in diagnosis and research. *Ultrastruct Pathol.* 2001; 25: 39-50.
31. Eyden B: The fibronexus in reactive and tumoral myofibroblasts: further characterisation by electron microscopy. *Histol Histopathol.* 2001; 16: 57-70.
32. De Wewer O, Mareel M. Role of tissue stroma in cancer cell invasion. *J Pathol.* 2003; 200: 429-447.
33. Hinz B, Gabbiani G. Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling. *Thromb Haemost.* 2003; 90 (in press).
34. Toti P, Tanganelli P, Schurfeld K, Stumpo M, Barbagli L, Vatti R, Luzi P. Scarring in papillary carcinoma of the thyroid: report of two new cases with exuberant nodular fasciitis-like stroma. *Histopathology.* 1999; 35: 418-422.
35. Law AM, Oliveri CV, Pacheco-Quinto X, Horenstein MG. Actin expression in purely nodular versus nodular-infiltrative basal cell carcinoma. *J Cutan Pathol.* 2003; 30: 232-236.
36. Crocker DJ, Murad TM. Ultrastructure of fibrosarcoma in a male breast. *Cancer.* 1969; 23: 891-899.
37. Dickersin GR. Diagnostic electron microscopy: a text/atlas. New York and Tokyo: Igaku-Shoin, 1988, 162-163.

38. Eyden B. Smooth-muscle type myofilaments and actin in reactive and neoplastic nonmuscle cells. *Ultrastruct Pathol.* 2000; 24: 347-352.
39. Nielsen GP, Keel SB, Dickersin GR, Selig MK, Bhan AK, Rosenberg AE. Chondromyxoid fibroma: a tumor showing myofibroblastic, myochondroblastic, and chondrocytic differentiation. *Mod Pathol.* 1999; 12: 514-517.
40. Shek TW, Peh WC, Leung G. Chondromyxoid fibroma of skull base: a tumour prone to local recurrence. *J Laryngol Otol.* 1999; 113: 380-385.
41. Suzuki S, Oka H, Tanaka R, Kawano N, Fujii K, Dobashi Y, Iwabuchi K. A chondromyxoid fibroma-like tumor of the cranial convexity: immunohistochemical and ultrastructural study. *Clin Neuropathol.* 1999; 18: 37-41.
42. Povysil C. Actin-positive chondroblasts (myochondroblasts) in benign chondroblastoma. *Cesk Patol.* 1995; 31: 77-78.
43. Reddick RL, Popovsky MA, Fantone JC 3d, Michelitch HJ. Parosteal osteogenic sarcoma. Ultrastructural observations in three cases. *Hum Pathol.* 1980; 11: 373-380.
44. Martinez-Tello FJ, Navas-Palacios JJ. The ultrastructure of conventional, parosteal, and periosteal osteosarcomas. *Cancer.* 1982; 50: 949-961.
45. Skalli O, Gabbiani G, Babai F, Seemayer TA, Pizzolato G, Schurch W. Intermediate filament proteins and actin isoforms as markers for soft tissue tumor differentiation and origin. II. Rhabdomyosarcomas. *Am J Pathol.* 1988; 130: 515-531.