Modulation of fibroblast activity by collagens
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Summary

Fibroblasts are the predominant cells in soft connective tissues and play a key role in the homeostasis of these tissues by continuously degrading and synthesising extracellular matrix components. A proper regulation of the metabolic activity of the fibroblast is thus a prerequisite for the maintenance of soft connective tissues in the organism. How modulation of the activity of fibroblasts is controlled is not exactly known. Although it is generally taken that growth factors and cytokines are crucial for this modulation, recent data suggest that extracellular matrix components (e.g. collagens) may play a role in this process as well. It was the aim of the studies presented in this thesis to elucidate the contribution of collagens in modulating fibroblast activity and behaviour. Special emphasis was put on the expression of an important class of enzymes involved in the breakdown of the extracellular matrix: the matrix metalloproteinases (MMPs).

By using soft connective tissue explants (periosteum of calvaria) it was found that gelatinase A (MMP-2) and not the collagenases (MMP-1, -8 and -13) were essential for the digestion of the collagen in the tissue (Chapter 2). Modulation of the activity and expression of MMP-2 proved to depend on MMP-activity including MMP-2 itself and not, as generally assumed, on the activity of growth factors and cytokines. If fibroblasts were isolated from the periosteal tissue, however, growth factors and cytokines (Chapter 3) could induce expression of MMP-2. Since these data suggested the involvement of extracellular matrix components in the modulation of fibroblast activity, in the following studies participation of different types of collagen (types I, III and V) was investigated (Chapters 4, 5, and 6).

In Chapters 4 and 5 it is shown that collagen type I, III and V modulate the expression and activation of MMP-1, -2 and -3 by fibroblasts. Of particular interest was the finding that low concentrations of collagen resulted in an increased expression and activation of MMP-2, whereas MMP-1 and -3 were down-regulated under these conditions. Higher concentrations of the collagens up-regulated all MMPs. Another intriguing finding was that, if compared to type I and III collagen, type V collagen had the weakest effect on the modulation of the various MMPs. Independent of the type of collagen, the induction of MMP-expression was mediated by the β1-integrin family and the intracellular signal depended on the activity of tyrosine kinases, protein kinase A, and protein kinase C (Chapters 4 and 5).

Since fibroblasts do not form a homogeneous population of cells it is possible that, in addition to differences in the composition of the tissue environment, also intrinsic differences play a role in their activity. In Chapter 5 it is demonstrated that different fibroblast subsets
periosteal, periodontal ligament (PDL) and gingival fibroblasts) reveal differences in the level of expression of MMPs, although the overall effects of the collagens are similar. Gingival fibroblasts expressed the highest level of activity of MMP-1 and MMP-3, whereas MMP-2 was highest in cultures of PDL fibroblasts. Comparing these data with collagen turnover data presented in the literature, support is given to the view that MMP-2 plays an important role in collagen breakdown under physiological conditions.

In addition to the effects of the collagens on MMP-expression, these components proved to modulate proliferation of fibroblasts (Chapter 6). Each of the three types of collagen dose-dependently stimulated cell proliferation. Whereas all concentrations of type I and III collagen stimulated cell proliferation, type V collagen dose-dependently up- or down-regulated proliferation. In spite of these different reaction patterns the involvement of various intracellular cascades proved to be similar. In all instances it appeared that tyrosine kinases and protein kinases were involved in collagen-stimulated cell proliferation.

The data presented in this thesis indicate that components of the extracellular matrix, among which type I, III and V collagen, may play an essential role in proliferation and the modulation of the activity of soft connective tissue cells. We propose that the regulation of fibroblast activity greatly depends on the amount and nature of the extracellular matrix constituents available to the cells. Under normal physiological conditions relatively low levels of collagens are freely accessible to fibroblasts, resulting in a relatively low level of proliferation and a low level of enzyme expression. If under certain conditions (e.g. damage of the tissue) part of the collagenous meshwork is disrupted and comes into contact with the cells, proliferation as well as enzyme expression would than increase.
Fibroblasten zijn de meest voornamelijk actieve soort in de blauwzwarte en spelen een belangrijke rol bij de kracht en verzet van deze weefsel. De cellen zijn verantwoordelijk voor de constante samenvoering (afbraak en synthese) van de componenten die de extracellulaire matrix vormen. Een goede regulatie van de natuurlijke activiteit van fibrilloblasten is cruciaal voor de (955) soorten van functies van een extracellulaire matrix (ECM).

Algemeen wordt aangenomen dat bij de processen geneeshealing en cytoplasmatische voornamelijk rol spelen. Daarom, vond worden dat deze processen onder alle omstandigheden zijn belangrijk, ondanks of niet. Recentelijk is aangegeven dat ook componenten van de extracellulaire matrix (ECM) en niet-collagene extracellular activities kunnen beïnvloeden. In dit proces wordt de rubber opstaan. Daarbij is het bijzondere scheidingsbood thaan de modulatie van een belangrijke groep van enzymen die betrokken zijn bij de afbraak van de extracellulaire matrix, de matrix metalloproteinasen (MMPs).

In de eerste twee hoofdstukken (4.1 en 4.2) werd genoteerd gebruik van blauwzwarte-explantaties (perifere van de aanwezigheid van fibroblasten) die oorspronkelijk zijn door een eigen matrix te bevorderen. Het onderzoek vond plaats aan de klinische A (MMP-2) van belang is bij de afbraak van collageen. De collageenases (MMP-1, 9, 13) daarmee duiden blauken bij de processen met of aanwezig zijn bij zijn (Hoofdstuk 2).

In Hoofdstuk 3 is het aangemerkt dat betrokken is bij de extracellulaire activering van MMP-2 in het explantaat-modell. Alhoewel er verondersteld wordt dat geenfactoren en cytokinen de modulatie van MMP-2 uitermate blauken vanwege een weefselremmende effecten van deze cytokinen op MMP-2. Een resultaat van deze cellen op de gen afbraak en cytoplasmatische wordt echter wel gewerkt na coronarie van de cellen die het weefsel. De resultaten worden verder aan dat MMPs, en in het bijzonder de ene en gene geneesfractie van deze enzymen, betrokken zijn bij de weefselremmende activering van MMP-2.

De bevindingen van Hoofdstukken 4 en 5 en menaam de activering van fibroblasten (bijv. modulatie van MMPs) en weefselremmend wezenlijk onder in 4.4 dat niet genoteerde cellen, aangegeven dat de extracellulaire matrix betrokken is bij de modulatie. In de
(periodontal, periodontal ligament (PDL) and gingival fibroblasts) revealed differences in the level of expression of MMPs, although the overall effects of the collagenase were similar. Gingival fibroblasts expressed the highest level of activity of MMP-1 and MMP-3, whereas MMP-2 was highest in cultures of PDL fibroblasts. Comparing these data with collagen turnover data presented in the literature, support is given to the view that MMP-2 plays an important role in collagen breakdown under physiological conditions.

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