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Role of fibronectin in normal wound healing

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Abstract

Fibronectin is an adhesive molecule that plays a crucial role in wound healing, particularly in extracellular matrix (ECM) formation and also in reepithelialisation. Fibronectin plays many different roles in the wound healing process because of the presence of specific function domains and binding sites in its structure. Fibronectin interacts with different cell types, cytokines and the ECM. The main role of fibronectin is ECM formation. First, plasma fibronectin forms a provisional fibrin–fibronectin matrix, which will later be replaced by the mature ECM-containing tissue fibronectin.

Introduction

Wound healing is a complex and dynamic process, which involves interaction between different cells and molecules (1). Normal wound healing consists of different stages: haemostasis, inflammation, proliferation and remodelling (2). An adhesive molecule known as fibronectin plays different roles in different stages of wound healing, with the main role being cellular adhesion (3,4). Other roles are mediation of cell growth and cell migration (2,5,6).

In the last two decades of the 20th century, there has been extensive research into the structure of fibronectin and the role of fibronectin in the extracellular matrix (ECM) in wound healing and tissue repair or formation (3,7,8). A number of recently published studies are available that examine the construction of ECM fibronectin in wound repair in vitro (9–11). This article reviews the role of fibronectin in ECM formation, especially in the proliferative phase, both in wound repair and tissue repair of other connective tissue.

Function and structure of fibronectin

Fibronectin is a large glycoprotein that is found in all kinds of tissues and is essential in many different cell–matrix interactions (12,13). All fibronectin molecules consist of the same basic functional domains. Although encoded by single genes, the sequence is subdivided into approximately 50 exons (3), exons being a piece of DNA in the gene (14). The fibronectin gene is composed of three general types of homologous repeating units or modules, termed types I, II and III. In the gene, each repeating module of the type I or II homology unit is encoded by a separate exon. In contrast, a type III requires the contribution of two exons each. These modular repeating units are used as building blocks and are organised into larger structural domains with distinct functions. Type I modules are used to bind fibrin, heparin or collagen. The type II modules are found only in the collagen-binding domain and the type III modules are used in the domain that binds to cells. Type III also has the possibility of alternative splicing. When the exons from genes are built in a different manner during RNA splicing it is called alternative splicing, which will result in different sequence patterns, leading to different variables that give different expressions of fibronectin (3,13,14). Because of the specific function domains and binding sites, fibronectin interacts with different cell types, cytokines and the ECM (15). Fibronectin binds to other cell surfaces by integrins. An integrin links the fibronectin outside the cell to the actin filaments inside the

Key Messages

- fibronectin is essential for tissue formation in both wound repair and connective tissue repair
- fibronectin is one of the building blocks in extracellular matrix formation
- most research studies of fibronectin were conducted in vitro and they investigated the binding properties of this molecule
- fibronectin interacts with other cells to form an ECM in all stages of wound healing
- the aim of this article is to assess the role of fibronectin in normal wound healing

cell. When parts of fibronectin molecules are bound together, they form a fibril (3,13).

There are two forms of fibronectin: plasma fibronectin, which is synthesised in a soluble form by hepatocytes into the blood plasma (4,16), and cellular or tissue fibronectin, which is produced by different cells such as fibroblasts, endothelial cells and keratinocytes (Hynes and Yamada 1982, cited in Ref. 17). Plasma fibronectin is more important in the early phase of wound healing where it binds to platelets and fibrin, resulting in providing increased strength to the fibrin clot (4). Moreover, fibronectin in this clot is important for various platelet functions such as adhesion, migration and aggregation. Later in wound healing, endothelial cells and fibroblasts enter the wound bed and deposit cellular fibronectin that is important for granulation tissue formation (4). These findings are in concordance with the findings of Clark (7) who demonstrated the role of fibronectin under the migrating epithelium in reepithelialisation. However, later work by Moretti *et al.* (18) suggested that the major part of tissue fibronectin is plasma-derived.

Role of fibronectin in ECM formation in the proliferative phase of wound healing

Fibronectin also plays a role in the early phases of wound healing. The first step in haemostasis after wounding is the release of platelets from the blood flow in order to form a platelet plug. Interplatelet binding occurs through integrins via fibrinogen (19). The fibrin clot is further stabilised by factor XIII, where this factor binds fibrins to fibronectin, to form a fibrin–fibronectin clot (20). Fibronectin has been detected in areas of active bleeding in humans following fatal trauma (21). In the inflammatory phase, fibronectin is able to opsonise ECM debris. Beyond that, it activates macrophages so that they can phagocytise the debris (15).

Early research identified the distribution of fibronectin during wound healing by creating rectangular wounds in pigs (22). Biopsies from the animals demonstrated that fibronectin was part of the fibrin clot observed on the surface of mononuclear cells (i.e. macrophages), and that collagen fibrils were covered with fibronectin. From these data, Grinnell *et al.* suggested that fibronectin formed the substratum for cell migration and that fibronectin was a major component during wound healing.

Furthermore, Clark *et al.* suggested that fibronectin – when cross-linked with fibrin – might play a role in epidermal cell migration in vivo (23). Donaldson and Mahan (24) also demonstrated that fibronectin and fibrinogen are mediators for epidermal keratinocytes to migrate through the provisional wound bed, showing the importance of fibronectin in wound healing. However Guo *et al.* (25) suggest that keratinocytes need to be activated by integrins before migration occurs. A later study by Donaldson *et al.* (26) attempted to localise the domain of fibronectin that mediates this migration. They demonstrated that new epidermal cell migration is restricted to a domain in the middle third of the gene. The results contrasted with the earlier study by Donaldson and Mahan (24), whose results suggested that cell migration was shared equally by all regions of the molecule. A subsequent study by Igisu (27) demonstrated the role of plasma fibronectin in reepithelialisation in wound healing in vivo. Labelled plasma fibronectin was detected in the wound just 3 hours after wounding, and more remarkably accumulation was visible after 48 hours. Furthermore, they demonstrated migration of new epithelial cells and precipitation of fibrous fibronectin in the upper papillary dermis 3 days after wounding. Based on these findings, Igisu suggested that plasma fibronectin plays an important role in migration and adhesion of epithelial cells in the reepithelialisation process.

Keratinocytes are capable of upregulating the fibronectin-binding integrins (28). Later on, this was also demonstrated by Ongenae and coworkers (29) by examining the levels of fibronectin mRNA and α5β1 integrin. They demonstrated that the level of fibronectin mRNA in acute wounds was similar to that found in normal skin. Integrin α5 was heavily induced throughout the epidermis of acute wounds. Together, these findings suggest that fibronectin in the provisional matrix enhances epithelialisation mediated by integrins.

Greiling and Clark (30) investigated that fibroblast migration from the periwound collagenous matrix into the wound bed was fibronectin dependent in vitro. They demonstrated that transmigration into the fibrin clot without fibronectin was decreased by about 80%, suggesting that fibroblast migration was fibronectin dependent in the fibrin clot provisional matrix (30). Furthermore, they also demonstrated that fibroblast migration was plasminogen dependent and that fibronectin had to be present in both the collagenous and the provisional matrix to facilitate the transmigration process. Clark (7) suggests that fibronectin is also synthesised by fibroblasts, which is one of the key cells involved in the proliferation phase, the main function being to synthesise the ECM (2).

Along with fibronectin, the ECM is made up of collagens, proteoglycans, laminin and tenascin (31). The synthesis of fibronectin and fibrogenesis is promoted by two important cytokines, namely, the transforming growth factor β and connective tissue growth factor (4). Singh *et al.* (32) identify that ECM formation requires the creation of fibrils via fibronectin molecules. Subsequently, the fibronectin matrix deposition in wounds stimulates collagen deposition and also contributes to wound contraction (8). Using integrin α5β1, fibronectin is able to bind to other cells to further stabilise the ECM (15). Moreover, fibronectin binds glycosaminoglycans such as heparin sulphate and hyaluronic acid, the promoters of wound healing (7).

Corbett *et al.* (33) compared fibronectin alone and fibronectin within a fibrin matrix in vitro and demonstrated that there was a different cytoskeletal response from attached cells (33). They also demonstrated that cell morphology and behaviour were dependent on the molecular context of the ECM. On the basis of these findings, they suggested that substrate composition may play a role in regulating cell shape and size. A decade later, Midwood *et al.* (34) investigated different mechanisms for modulating cell responses to fibronectin matrix in vitro. They studied the complex interplay between adhesion, adhesion modulation and matrix turnover during tissue repair. Their results suggested that tenacin-C prevented premature wound contraction. They also demonstrated that α4β1 plays a role in fibronectin binding

during granulation tissue formation. In normal wound healing, fibroblasts adhere to fibronectin via α5β1 integrin receptors when entering into the fibrin–fibronectin provisional matrix (35). The provisional matrix contains mostly plasma-derived fibronectin (4). This plasma fibronectin contains predominantly subunits for α5β1 integrin binding in contrast to tissue fibronectin that contains more subunits with a binding site for α4β1 (34). When the provisional matrix is degraded and replaced by granulation tissue, fibronectin fragments bind to α4β1. However, for this binding, α4β1 needs to be activated and this activation can be achieved by exogenous treatment, in this case Mn^{2+}. This in vitro study highlighted several ways of modulating fibronectin matrix interaction with cells in tissue repair. This is important because accumulation of fibronectin-rich fibrillar matrix will stimulate further matrix deposition.

A review by Singh *et al.* (32) outlined the composition of fibronectin and ECM. They suggested that focal adhesion kinase (FAK) is mandatory for integrin binding. FAK is also needed for fibroblast assembly of fibronectin fibres as well as to maintain matrix association with the cell surface. When a fibril grows, the matrix matures. During that phase the matrix is converted into an insoluble form. Fibronectin matrix assembly is a dynamic and continuous process. Continuous polymerisation of fibronectin is essential in order to stabilise the matrix to the cell surface. Other ECM proteins that depend on fibronectin during their assembly are elastic fibres and type I collagen. Because of the ability of different splicing, Singh *et al.* (32) concluded that current understanding of the requirements for all of these binding sites is limited because of differences in the experimental approach that yielded different interpretations of function. Whilst Singh *et al.* produced an excellent review of the function of fibronectin, it is difficult to translate some of this to clinical practice. This might indicate that all the research performed so far was focussed on the exposure of the complex structure and of the different functions of this adhesive molecule.

ECM remodelling is a complex and regulated process and is necessary for regulation of cell migration (10). From their in vitro research in fibronectin polymerisation, Sottile and Hocking (9) demonstrated that polymerisation of fibronectin into the ECM leads to composition and stability of the ECM and to cell-matrix adhesion (9). Fibronectin polymerisation promotes collagen types I and III deposition into the ECM and regulates the turnover and endocytosis of ECM collagen I, which results in stabilisation of collagen I matrix fibrils (11). A study by Shi and Sottile (36) demonstrated that membrane-type matrix metalloproteinase 1 (MT1-MMP/MMP 14) promotes the turnover of ECM fibronectin by regulating the cleavage of large fibronectin fibrils and subsequently regulates endocytoses of the α5β1 integrin. Furthermore, they showed that inhibiting fibronectin polymerisation accelerates myofibroblast migration (36).

Although these studies demonstrated the important role of fibronectin in ECM remodelling, they are all only in vitro studies. The role of fibronectin in vivo has not been assessed. Therefore, it has not been possible to correlate these findings to human wound healing, yet.

Role of fibronectin in clinical practice

Following a number of research studies, the role of fibronectin in wound healing is becoming clear. In normal wound healing, its role is visible in all phases. The levels of plasma fibronectin are critical in haemostasis (18). After wounding it is visible that bleeding stops and that a clot is formed. By promoting aggregation of platelets and adhesion to the damaged endothelial surface, fibronectin gives strength to the clot by forming a fibrin–fibronectin network, also called the fibrin–fibronectin provisional matrix (15,19). This prevents wounds from subsequent bleeding. Although not always visible in clinical practice, fibronectin plays a role in inflammation by promoting macrophage activation and opsonising debris (1,15).

In the proliferation phase, the provisional matrix and particularly fibronectin is the main conduit for cell migration. About 4 days after wounding, fibroblasts invade the provisional matrix, followed by the endothelial cells (2,37). Fibroblasts produce matrix proteins for new ECM formation and the endothelial cells promote neovascularisation (1,2). Together, the new matrix and capillaries form a reddish, shiny hyperaemic granulation tissue that is clearly visible (2). Indirectly, fibronectin mediates wound contraction by providing a scaffold to which both myofibroblast and collagen fibrils can adhere to and contract (38). In clinical practice, this is demonstrated by a difference in original wound size and the final size of the scar that is much smaller. The decrease in wound size is approximately 40% (38).

Lastly, when the scar matures, fibronectin is broken down to create place for collagen deposition, which gives strength to the final scar (2,38).

Epithelialisation occurring during all phases of wound healing is probably the most visible action in wound healing and during which new epithelial cells migrate into the wound bed owing to the fibronectin provisional matrix (7,8).

Conclusion

The role of ECM fibronectin in regulating cell proliferation and cell adhesion and deposition of fibronectin into the ECM is difficult to assess (39). This article has highlighted that fibronectin plays a crucial role in ECM formation and also in reepithelialisation. Although fibronectin has many roles, Donaldson and Mahan (24) have only been able to demonstrate part of the picture, and therefore further research is required. However, it is clear that fibronectin interacts with other cells to form an ECM in all stages of wound healing. In addition, the role of fibronectin in the provisional matrix is better established than the mature ECM. However, most of the work on the mature ECM was in vitro and fibronectin behaves differently in wounds in vivo.

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