6 The Instable Scar

R. Rosch, M. Binnebösel, K. Junge, P. Lynen-Jansen, P.R. Mertens, U. Klinge, V. Schumpelick

Introduction

There is increasing evidence that inguinal hernia formation is based on a disorder of the connective tissue biology [1–12]. Similarly, secondary herniations as incisional hernias or diverse recurrent hernias are supposed to be associated with biological factors that provoke an instable scar formation during the wound-healing process. Patients with congenital connective tissue disorders have a higher risk of developing of incisional or recurrent hernias [13, 14]. Furthermore, aneurismal disease, as another collagen disorder, has repeatedly been shown to be associated with an increased risk for the development of incisional hernias [15–17]. Previous work was able to support the hypothesis of a dysregulation of the collagen metabolism in patients with incisional hernias. In patients with either primary or recurrent incisional hernias, a significantly decreased ratio of collagen type I to type III and alterations of collagen-interacting proteins were found [18–21]. An alteration of the collagen composition was further verified by a comparative immunhistochemical analysis of surgical mesh explants where patients operated for hernia recurrence had a lowered collagen I/III ratio as compared to patients operated due to mesh infection or mesh-related pain [22].

In order to understand what leads to this altered connective tissue quality in (recurrent) incisional hernia patients, we performed an immunohistochemical characterization of factors with potential impact on the wound-healing process in comparison to non-hernia patients.

Materials and Methods

Abdominal skin scars from patients with recurrent incisional hernias were excised in the course of hernia repair. Abdominal skin scars of patients without any history or clinical evidence of hernia who underwent relaparotomy due to diverse intra-abdominal diseases served as controls. Patients under steroid therapy, extraordinary obesity (body mass index > 35) or history of connective tissue diseases were excluded from the study.

Immunohistochemical and cross polarization microscopy studies were performed with paraffin embedded tissue sections. The following primary antibodies were applied: catenin, c-myc, factor XIII, notch, SMA, ESDN, TGF-β, PAI, uPAR, YB-1, COX-2, p53. Collagen-I/III ratios were analyzed as described previously [21]. The expression of immunohistochemical parameters was analyzed by an immunoreactive score (IRS) where the score ranges from 1 to 20 [23].

In order to characterize the functional network of the investigated parameters, associations between variables were calculated through two-sided Spearman correlation test within each group. Associations between variables were assumed with p values < 0.05 and shown graphically. Additionally, linkages between parameters were calculated by the clustering coefficient c (see Fig. 6.1):

$$c = \frac{E}{K(K-1)/2}$$

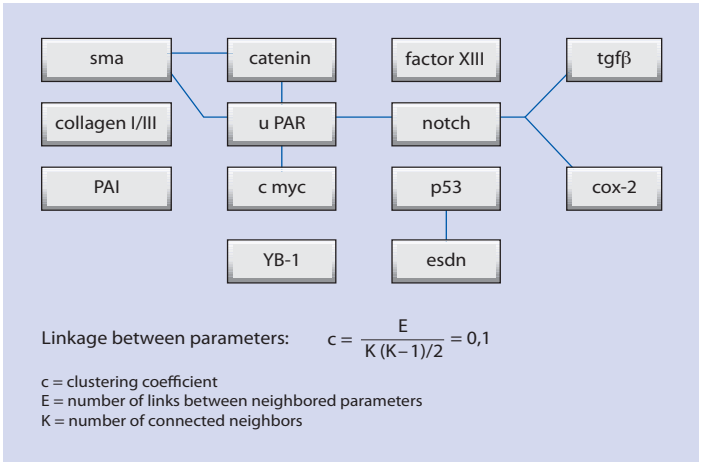
Results

Functional interferences of the matrix parameters within the postulated network were assessed by two-sided Spearman correlation analyses. ◘ Figures 6.1 and 6.2 show the graphic representation of this network with related parameters linked by bars (significant correlation coefficient with $p < 0.05$). In summary, we found differences in association patterns of matrix parameters between skin scars from recurrent hernia and from control patients. In the recurrent hernia group associations were found for collagen I/III with factor XIII and uPAR, PAI and c-may and TGF-β and COX-2. In the control group there were associations between SMA and catenin, ESDN and p53, notch and TGF-β and COX-2 and between uPAR and c-myc, notch, catenin and SMA.

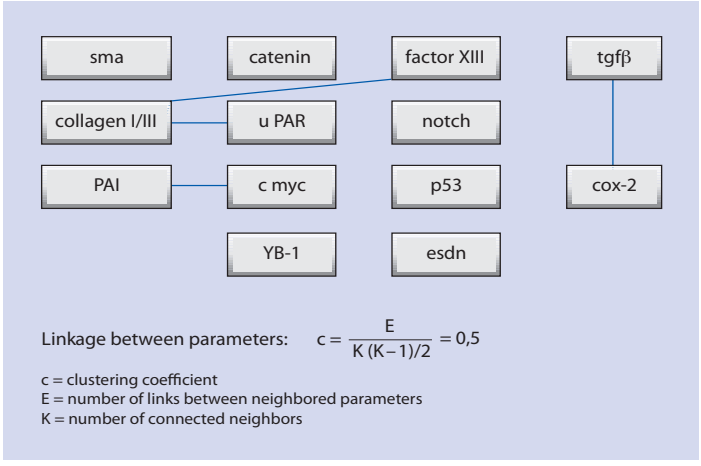
Calculated cluster coefficients (c) within the two networks also showed pronounced differences between both groups with a higher degree of crosslinking in controls ($c = 0.1$) as compared to recurrent hernia ($c = 0.05$).

Discussion

Wound healing and scar formation are tightly regulated and highly dynamic and complex processes characterized by permanent cell turnover and matrix remodelling. The modulation of this network is influenced by the interplay of numerous cellular and extracellular factors, hereby determining the quality of scar formation. Already mild disturbances of this



◘ **Fig. 6.1.** Spearman correlations – control scar



◘ **Fig. 6.2.** Spearman correlations – recurrent hernia scar

system may thus predispose to insufficient scar formation, hereby leading to incisional or recurrent hernia formation. For inguinal hernia recurrence, which might be regarded as a subtype of incisional hernia showing with similar alterations of collagen quality, Sorensen et al. have shown that smoking is an important risk factor, presumably due to an abnormal connective tissue metabolism in smokers [24, 25].

Previous investigations about the collagen-interacting proteins in scar tissue from patients with incisional hernias showed divergent expression patterns for the matrix-metalloproteinase MMP-1 and the discoidin domain receptor DDR-2 when compared to controls [21]. Additionally, in in vitro studies about fibroblast function in patients with recurrent incisional hernias we found a specific cell response after biomaterial contact as compared to control fibroblasts [26].

These results indicated an altered remodelling and phenotype in a population at risk that might be regarded as additional causative factors for a defective scar formation. However, focusing on single alterations of expression profiles does not reflect the complex cross talk within the cellular and extracellular matrix network during wound healing. With the analyses of correlations and clustering of diverse parameters with known impact on cell-cell adhesion and interaction, migration, angiogenesis, cell differentiation and proliferation we thus tried to map the scar architecture in patients with recurrent incisional hernias as compared to controls. Here, the different associations between matrix parameters and respective clustering coefficients indicate a different intercommunication within the (cellular and extracellular) matrix in recurrent incisional patients that possibly is responsible for a defective scarring process.

Conclusion

In addition to a modified cell function our results indicate a disturbed intercommunication and a comprehensive change of matrix composition and turnover within the scar tissue in recurrent incisional hernia patients as a potential cause for the development of an insufficient scar formation. Further studies are needed for the understanding of the complex functions of this biological network.

Acknowledgements. We are grateful to Mrs. Ellen Krott for most excellent and careful assistance during this investigation.

References

1. Liem MS, van der Graaf Y, Beemer FA, van Vroonhoven TJ. Increased risk for inguinal hernia in patients with Ehlers-Danlos syndrome. Surgery 122 (1997) 114–115
2. Uden A, Lindhagen T. Inguinal hernia in patients with congenital dislocation of the hip. A sign of general connective tissue disorder. Acta Orthop Scand 59 (1988) 667–668
3. Pleumeekers HJ, De Gruijl A, Hofman A, Van Beek AJ, Hoes AW. Prevalence of aortic aneurysm in men with a history of inguinal hernia repair. Br J Surg 86 (1999) 1155–1158
4. Wagh PV, Read RC. Defective collagen synthesis in inguinal herniation. Am J Surg 124 (1972) 819–822
5. Conner WT, Peacock EE Jr. Some studies on the etiology of inguinal hernia. Am J Surg 126 (1973) 732–735
6. White HJ, Sun CN, Read RC. Inguinal hernia: a true collagen disease. Lab Invest 36 (1977) 359
7. Ajabnoor MA, Mokhtar AM, Rafee AA, Taha AM. Defective collagen metabolism in Saudi patients with hernia. Ann Clin Biochem 29 (Pt 4) (1992) 430–436
8. Bellon JM, Bujan J, Honduvilla NG, Jurado F, Gimeno MJ, Turnay J, Olmo N, Lizarbe MA. Study of biochemical substrate and role of metalloproteinases in fascia transversalis from hernial processes. Eur J Clin Invest 27 (1997) 510–516
9. Klinge U, Zheng H, Si ZY, Bhardwaj R, Klosterhalfen B, Schumpelick V. Altered collagen synthesis in fascia transversalis of patients with inguinal hernia. Hernia 3 (1999) 181–187
10. Klinge U, Zheng H, Si Z, Schumpelick V, Bhardwaj RS, Muys L, Klosterhalfen B. Expression of the extracellular matrix proteins collagen I, collagen III and fibronectin and matrix metalloproteinase-1 and -13 in the skin of patients with inguinal hernia. Eur Surg Res 31 (1999) 480–490
11. Klinge U, Zheng G, Si ZY, Schumpelick V, Bhardwaj R, Klosterhalfen B. Synthesis of type I and III collagen, expression of fibronectin and matrix metalloproteinases-1 and -13 in hernial sac of patients with inguinal hernia. Int J Surg Investig 1 (1999) 219–227
12. Rosch R, Klinge U, Si Z, Junge K, Klosterhalfen B, Schumpelick V. A role for the collagen I/III and MMP-1/-13 genes in primary inguinal hernia? BMC Med Genet 3 (2002) 2
13. Girotto JA, Malaisrie SC, Bulkely G, Manson PN. Recurrent ventral herniation in Ehlers-Danlos syndrome. Plast Reconstr Surg 106 (2000) 1520–1526
14. McEntyre RL, Raffensperger JG. Surgical complications of Ehlers-Danlos syndrome in children. J Pediatr Surg 12 (1977) 531–535
15. Augestad KM, Wilsgaard T, Solberg S. Incisional hernia after surgery for abdominal aortic aneurysm. Tidsskr Nor Laegeforen 122 (2002) 22–24
16. Adye B, Luna G. Incidence of abdominal wall hernia in aortic surgery. Am J Surg 175 (1998) 400–402
17. Papadimitriou G, Pitoulias G, Papaziogas B, Koutsias S, Vretzakis G, Argiriadou H, Papaziogas T. Incidence of abdominal wall hernias in patients undergoing aortic surgery for aneurysm or occlusive disease. Vasa 31 (2002) 111–114
18. Klinge U, Si ZY, Zheng H, Schumpelick V, Bhardwaj RS, Klosterhalfen B. Abnormal collagen I to III distribution in the skin of patients with incisional hernia. Eur Surg Res 32 (2000) 43–48

19. Klinge U, Si ZY, Zheng H, Schumpelick V, Bhardwaj RS, Klosterhalfen B. Collagen I/III and matrix metalloproteinases (MMP) 1 and 13 in the fascia of patients with incisional hernias. J Invest Surg 14 (2001) 47–54
20. Si Z, Rhanjit B, Rosch R, Rene PM, Klosterhalfen B, Klinge U. Impaired balance of type I and type III procollagen mRNA in cultured fibroblasts of patients with incisional hernia. Surgery 131 (2002) 324–331
21. Rosch R, Junge K, Knops M, Lynen P, Klinge U, Schumpelick V. Analysis of collagen-interacting proteins in patients with incisional hernias. Langenbecks Arch Surg 387 (2003) 427–432
22. Junge K, Klinge U, Rosch R, Mertens PR, Kirch J, Klosterhalfen B, Lynen P, Schumpelick V. Decreased collagen type I/III ratio in patients with recurring hernia after implantation of alloplastic prostheses. Langenbecks Arch Surg 389 (2004) 17–22
23. Stegner HE, Remmele W. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 8 (1987) 138–140
24. Zheng H, Si Z, Kasperk R, Bhardwaj RS, Schumpelick V, Klinge U, Klosterhalfen B. Recurrent inguinal hernia: disease of the collagen matrix? World J Surg 26 (2002) 401–408
25. Sorensen LT, Friis E, Jorgensen T, Vennits B, Andersen BR, Rasmussen GI, Kjaergaard J. Smoking is a risk factor for recurrence of groin hernia. World J Surg 26 (2002) 397–400
26. Rosch R, Lynen-Jansen P, Junge K, Knops M, Klosterhalfen B, Klinge U, Mertens PR, Schumpelick V. Biomaterial-dependent MMP-2 expression in fibroblasts from patients with recurrent incisional hernias. Hernia 10 (2006) 1–6

Discussion

Franz: *There are some recurrences so early so that there has to be a mechanical surgical component and yet groups like your own are demonstrating clear biological effects in these complicated patients. I have two questions: can you predict who is going to develop an incisional hernia? If you had a pre-operative biopsy, are you able to predict who, after the laparotomy, will develop an incisional hernia? Obviously that would be the ultimate goal. And finally do you have any idea how possibly a failed wound might lead to a systemic change? I personally think that a lot of early surgical failures are the mechanism behind these incisional hernias and yet you are demonstrating a systemic effect. Are you able to predict prospectively who develops a hernia and have you any idea how maybe an early laparotomy wound might induce changes you are seeing in the skin?*

Rosch: *With regard to the first part of the question I hope that we will in future have a test and Dr. Mertens has told us already that he will talk about this part. It might be possible, but maybe not for all hernia patients because there are also the technical reasons that have nothing to do with a pathological scar formation or the collagen metabolism. So we have to separate the patients at risk from technical reasons and also from other factors like smoking or medication. We still have to study a lot to understand the system and it is still quite unclear where to focus on – maybe on different parts of this network structure.*

Franz: *But the skin of all these patients has healed despite the measurement of a matrix disorder?*

Rosch: *Yes, the skin is easy to investigate; of course, it would be better to investigate the fascial structures. But what we found in the fascial structures before with regard to the collagen type-I/III ratio was the same as in the skin.*

Kehlet: *I totally agree with you regarding incisional hernias. But what about the inguinal hernias? Do you think that this is important in inguinal hernias and is it going to replace a sufficient surgical technique or should we simply not consider this for inguinal hernias?*

Rosch: *You have to separate the primary hernias from the recurrent inguinal hernias, which in my opinion are similar to incisional hernias with regard to their pathogenesis – they are also a kind of incisional hernia.*

Kehlet: *But you see the problem with these series with no recurrences so maybe it is a question of surgical technique and not of the collagen problem in inguinal recurrences?*

Rosch: *If you have a mesh structure in your inguinal region it is more difficult to develop a recurrence.*

Kingsnorth: *I think we must separate the two problems. Dr. Read was talking about direct herniation which is a primary phenomenon of collagen. We must separate this from the patients with wound failure, which is incisional hernia. These patients have had their fascia disturbed and it is a failure of the wound rather than a primary failure of the fascia. So I think it is certain that there are differences between these two mechanisms and we should not confuse the two.*

Rosch: *Of course not. But the patients who will develop primary hernias also more often develop secondary hernias, for example incisional hernias. The reason why the collagen type-I/III ratio is disturbed might be different in the secondary as compared to the primary hernias. But the problem remains the same.*

Kingsnorth: *Yes, I agree, because we have just seen the data this morning that show that patients with direct hernias have a higher instance of recurrence. This is probably because they already have a primary phenomenon that caused the direct hernia but in addition they may have a secondary phenomenon which is scar failure, wound failure itself. But I think that these are two separate mechanisms.*

Rosch: *Yes, possibly different factors that are combined.*