Expression of Urokinase-type Plasminogen Activator and Its Receptor in Keloids

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Background: The urokinase-mediated plasminogen activation (uPA) system plays a central role in a number of cellular processes including tissue remodeling, cell migration, and angiogenesis. Elevated uPA activity has also been seen with tumor invasion and metastasis in a variety of malignancies. Keloids represent an aberrant form of wound healing characterized by uncontrolled growth with invasion beyond the margins of the original wound. The regulation of this cellular process remains poorly understood. We hypothesize that keloids will have increased staining percentage for uPA and its receptor (uPAR) compared with normal scars. To our knowledge, no previous studies have examined the relationship of uPAR in keloid formation.

Design: Analysis of uPAR expression by immunohistochemistry in paraffin sections from 20 keloids and 18 normal scars. Expression was graded by a dermatopathologist according to percentage of cells staining for uPAR.

Setting: University Medical Center (Division of Otolaryngology–Head and Neck Surgery) and the Department of Dermatology at the University of Rochester Medical and Dental School, Rochester, NY.

Results: Of the 20 keloids, 8 (40%) strongly expressed uPAR (>50% of cells), while only 4 (22%) of 18 normal scars had similar staining. More than half of the normal scars stained minimally for uPAR (<5% staining). There was a strong expression of uPAR in the extracellular matrix in 14 (70%) of the 20 keloids, while no scar showed uPAR in the extracellular matrix.

Conclusion: Our observation suggests that the uPA system is involved in the expansion of keloids beyond the wound margins in part through the degradation of the extracellular matrix, a finding that is supported by the strong expression of uPAR in the extracellular matrix and collagenous cords in most keloids studied.


Keloids are unsightly lesions resulting from aberrant wound healing in predisposed individuals. They occur more commonly in darker pigmented patients with a predilection for the earlobe, chest, and neck. By definition, a keloid extends beyond the wound margins and can result in disfigurement, contraction, erythema, pruritus, and pain. The cellular regulation, or dysregulation of this process is poorly understood. Despite combined therapy with intralesional steroid and surgical excision, keloids maintain a high recurrence rate. Other keloid treatment modalities are largely empirical and anecdotal. There are many theories of keloid pathogenesis, prompting a multitude of studies. Consistent among these theories is a common observation: excessive collagen deposition in the dermis secondary to trauma or surgical injury. For a keloid to spread it must influence collagen deposition beyond its boundary. This requires alteration of the extracellular matrix (ECM). This complex process requires cell recruitment induced by molecular signaling pathways and communication between different cell types. Various proteolytic enzymes including the matrix metalloproteinase system and the plasminogen activation system are involved. The urokinase plasminogen activator (uPA) system and its receptor (uPAR) function in mediating and regulating cell recruitment, cell migration, cell-to-cell adhesion, and angiogenesis. On the cell surface, uPA, secreted as an inactive precursor (pro-uPA), binds to its receptor. Binding to the receptor accelerates the conversion of pro-uPA to active uPA. The active uPA rapidly converts the inactive plasminogen to plasmin, which helps regulate basement membrane and ECM degradation. Active uPA...
al results in the activation of other procollagenases and latent growth factors. By localizing the uPA to its receptor, enzyme activity can be localized to the leading edge of cell migration and at cell-cell contact sites of many cell types. Two plasminogen activator inhibitors (PAI-1 and PAI-2) help regulate the enzymatic activity of uPA. When active uPA is bound to its receptor, subsequent PAI-1 binding results in internalization and degradation of the complex. High levels of uPA, uPAR, and PAI-1 are important negative prognostic criteria in many cancers. Expression of uPAR antagonists has a drastic effect on the metastatic potential of cancer cells. To our knowledge, the expression of uPAR system has not previously been studied in keloids.

The present investigation examined expression of uPAR in both keloid and normal scar specimens using established immunohistochemistry techniques. We hypothesized that keloids behave similarly to tumors with uncontrolled local growth and extension beyond wound margins. We anticipated that keloids would have increased expression of uPAR compared with normal scars.

**METHODS**

Twenty histologically diagnosed keloids and 18 excised normal scars of various ages (13 days to >365 days) were identified from the dermatopathology database at the University of Rochester, Rochester, NY (Figure 1 and Figure 2). All specimens had paraffin blocks available for analysis. The investigators were blinded to all other clinical data prior to grading uPAR expression.

Immunohistochemical assays localized uPAR expression in tissue sections using a monoclonal antibody against human uPAR (No. 3936; American Diagnostica Inc, Greenwich, Conn) at a dilution of 1:30. Formalin-fixed sections of 4 μm were deparaffinized in xylene and rehydrated in graded ethanol solutions. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol. A diluted blocking serum (10% goat serum) was incubated for 30 minutes and then rinsed with phosphate-buffered saline. The primary antibody was applied and placed in an incubator for 60 minutes. Sections were then washed 3 times for 5 minutes in phosphate-buffered saline, and biotinylated goat anti-mouse IgG was added for 30 minutes. A standard avidin-biotin technique was then used (Vectastain Elite Murine ABC kit; Vector Laboratories, Burlingame, Calif) to demonstrate the antigens. Diaminobenzidine tetrachloride was used as the chromogen. All incubations were carried out in a humidified chamber at 37°C. After counterstaining with hematoxylin-eosin, sections were evaluated by light microscopy. For negative controls, the same protocol minus the primary antibody was followed.

The senior authors of this article (an experienced dermatopathologist [G.S.] and an otolaryngologist [T.D.D.]) experienced in immunohistochemical technique) independently graded uPAR expression. For each slide, the percentage of positive staining cells was estimated using the following divisions: less than 5%, 5% to 50%, and greater than 50%. The presence or absence of staining of the ECM was also recorded. A statistical comparison of staining patterns was made between keloid and scar using a χ² test. Statistical significance was defined as P < .05.

RESULTS

The pattern of uPAR expression in keloids and in scars was variable; however, receptor staining was primarily identified in tissue (fibroblasts) and in the stromal matrix. Expression of uPAR in keloids was greater than 50% in 8 (40%) of 20 (Figure 3A), 5% to 50% in 7 (35%) of 20 (Figure 3B), and less than 5% in 5 (25%) of 20 (Figure 3C). In normal scars, uPAR expression was greater than 50% in 4 (22%) of 18 (Figure 4A), 5% to 50% in 4 (22%) of 18 (Figure 4B), and 10 (55%) of 18 stained less than 5% (Figure 4C). All controls showed no staining for uPAR. These results are summarized in Figures 1 and 2.

A 3-way χ² test showed no significant difference in uPAR expression between the scars and keloids (P = .15). A post hoc analysis that grouped the data as negative (<5% uPAR expression) and positive (≥5% uPAR expression) also failed to achieve statistical significance (P > .05). We found a significant difference in ECM uPAR expression between keloid specimens and normal scars: 14 of 20 keloid specimens stained strongly, while none of the scars had staining in the ECM.

The expression of uPAR compared with scar age was also analyzed: 10 (56%) of 18 scars were less than 60 days...
old, 4 scars (22%) were greater than 60 days old, and 4 scars (22%) were of unknown age. Of the 10 immature scars, 4 stained strongly (>50%), 1 stained moderately, and 5 stained weakly for uPAR. None of the 4 mature scars stained strongly for uPAR (Figure 2).

**COMMENT**

Understanding the biology of keloids challenges researchers and frustrates clinicians. Despite numerous investigations, no single common pathway explains keloid formation and growth. The dysregulation in keloid growth has been demonstrated in each of the wound healing phases: (1) inflammation, (2) proliferation, and (3) maturation. During the inflammatory phase, there is a dramatic increase in cellularity. Neutrophils predominate during the first 24 to 48 hours and decrease over several days. Increased expression of inflammatory phase cytokines have been noted in keloids. Some of the keloids we studied were immature and still in the inflammatory phase of wound healing. The proliferative phase entails production and migration of fibro-
bects and their products. These cells produce the collagen, which in keloids shows aberrant cross-linking. The remodeling phase of wound healing demonstrates dysregulation in collagen lysis, epithelialization, and wound contraction with failure of apoptosis and decreased collagenase activity.

As with many immunohistochemical techniques, there is no standardization for reporting uPAR expression. A number of different grading scales have been used. We reported our results using a modification of the work by Weidle et al. Although the presence of a receptor does not directly reflect enzyme activity, it can serve as an indirect indicator. For our study, we sought to determine that keloids did indeed express the receptor for uPAR and at what concentration. Archived tissue was used as a preliminary study to detect the presence of this receptor. We observed greater staining in keloid cells and the ECM of the keloids compared with normal scars. There are no other data of uPAR expression for keloids with which to compare our findings. To determine actual uPA activity would require fresh tissue samples. Our results are limited to some degree by our sample size and incomplete data on scar age. Further studies are needed to confirm our observation.

We observed a greater degree of uPAR expression in keloids than normal scars. Interestingly, some immature scars also stained strongly. This result is not entirely unexpected. Immature scars are likely to be in the inflammatory stage of the wound healing where the cells known to express uPAR are more numerous. The interaction between uPAR and neutrophils has previously been observed; uPAR has reacted strongly with tissues that have a high neutrophilic population. The uPA receptor is present in many tissues and has been identified on monocytes, neutrophil granulocytes, leukocytes, endothelial cells, macrophages, fibroblasts, and cancer cells. We were initially blinded to scar age in the present study; however, in the retrospective review of the clinical histories, we chose to define immature scars as less than 60 days old. This age was based on previous work by Levenson et al., who found that the maximum strength achieved is approximately 80% that of the unwounded skin tests by 60 days. Interestingly, the only scars that exhibited complete 100% staining were those scars less than 60 days old. None of the more mature scars, albeit few in number, stained strongly positive. Further studies are needed to compare more mature scars and keloids.

It also needs to be noted that our analysis of keloids was of the excised pathologic specimen. We did not specifically examine the leading edge of the keloid where one would expect the process of ECM degradation to be most active. It is likely that uPAR expression would be highest in these leading edges of keloids. The ECM of the keloids stained strongly in most specimens, which would support the above theory. Normal scars showed no increased in staining of its ECM. This is believed to be the most positive finding in this study.

Another potential confounder to our finding is a lack of data about prior treatment. Because of the retrospective nature of the study, clinical details about prior treatments including interlesional and topical treatments were not available. We speculate that if the keloids were bothersome enough to warrant excision, some keloids were likely to have received treatment, thus potentially influencing uPAR expression. One would expect that those keloids with a poor response to topical and injection therapy may have a different pattern of uPAR expression.

CONCLUSIONS

This study demonstrates the expression of uPAR in keloids. Poorly regulated involvement of the uPA system is a potential pathway for the expansion of keloids beyond the wound margins through the degradation of the ECM. Confirming the participation of the uPA system in keloid development offers a promising opportunity: uPAR can be selectively inhibited by using a blocking antibody, thereby regulating the expansion of keloids. A greater understanding of the uPA-uPAR system in keloid development thus offers a potential opportunity to predict behavior and possibly direct treatment.

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