Upregulation of Cytokines Midkine and Pleiotrophin in Keloids

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INTRODUCTION

Keloids are benign proliferative scars that are exaggerated responses to cutaneous wound healing. They can be painful and/or pruritic, and commonly affect the chest, upper back, shoulders, and earlobes. Keloids occur only in humans; they have no known gender bias but often affect skin of color patients, especially those of African, Hispanic, or Asian descent. Though they are benign growths, prior research has shown that keloid fibroblasts display bioenergetics of cancer cells[4]. This project aims to identify novel molecules upregulated in keloids in order to identify uncharacterized mechanisms underlying keloid pathogenesis.

METHODS

Matched sets of keloid tissue and perilesional normal tissue were obtained from three keloid patients recruited from the outpatient dermatology clinics of Parkland Hospital and UT Southwestern Medical Center after IRB approval and informed consent was obtained (**Figure 1**). The gene expression in keloid tissue was compared to perilesional normal tissue using whole transcriptome sequencing. The data were filtered to target genes upregulated or downregulated by at least three-fold. A total of 344 genes showed differential expression; of note were Midkine (MDK) and Pleiotrophin (PTN), upregulated 30-fold and 10-fold respectively (**Table 2**).

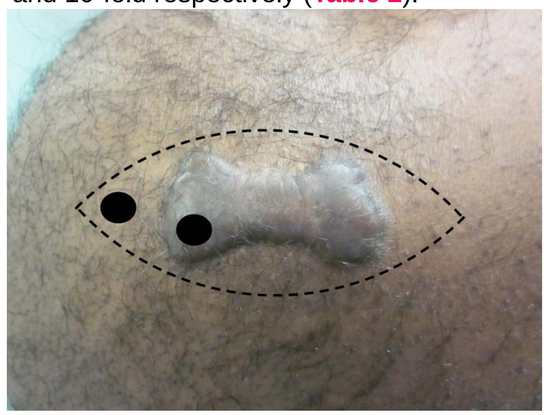

Figure 1: Perilesional tissue (outer area) and keloid tissue (inner area) were compared through whole transcriptome sequencing

METHODS/RESULTS

Whole Transcriptome Sequencing

Gene	Normal	Keloid	Log_2(fold change)	Test Statistic	P-value	Q-value	Significant
MDK	10.1213	278.434	4.78186	5.03085	5.00E-05	0.004493	Yes
PTN	22.1256	184.065	3.05643	3.90551	5.00E-05	0.004493	Yes

Table 2: Whole Transcriptome Sequencing Data for Midkine (MDK) and Pleiotrophin (PTN)

Midkine (MDK) and Pleiotrophin (PTN) are secreted cytokine signaling molecules known to play a role in wound healing, mitogenicity, and inflammation and are often upregulated during embryogenesis[1]. They are upregulated in several cancers, and higher expression generally indicates poor prognosis[2]. Because keloid fibroblasts display similar bioenergetics to that of cancer cells, we wanted to further investigate the roles of MDK and PTN in keloids.

Immunohistochemistry

To confirm the presence of MDK in keloid tissue, we performed immunohistochemistry staining for anti-MDK goat antibody (**Figures 3 and 4**).

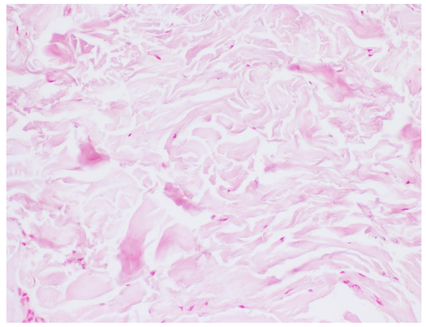

Figure 3: Perilesional normal skin tissue IHC stained with anti-MDK goat antibody

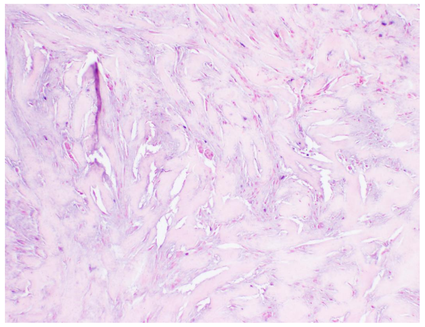

Figure 4: Keloid skin tissue IHC stained with anti-MDK goat antibody

Real-Time Polymerase Chain Reaction (RT-PCR)

We hypothesized that MDK and PTN would be upregulated in primary keloid fibroblasts *in vitro* versus normal skin fibroblasts *in vitro*. We performed RT-PCR on RNA obtained from 3 sets of keloid primary fibroblasts and normal primary fibroblasts grown *in vitro*, using MDK, PTN, and GAPDH (positive control) primers, and water serving as a negative control (**Figure 5**).

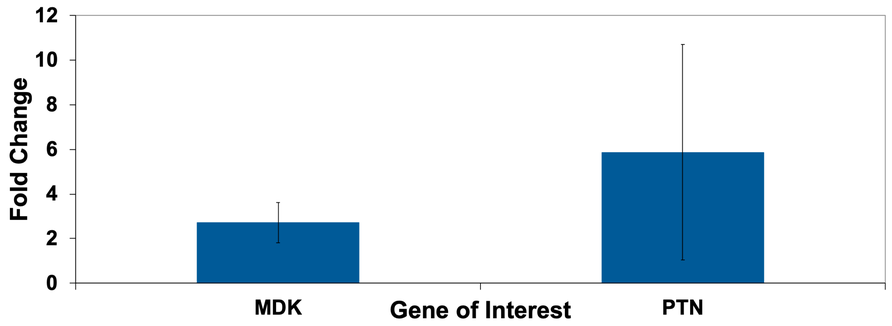

Figure 5: Graph displaying fold change between keloid primary fibroblasts and normal primary fibroblasts

DISCUSSION

The upregulation of *MDK* and *PTN* in the primary cells was not as high as that seen in whole tissue, It is possible that some other component of the keloid microenvironment, such as keratinocytes, endothelial cells, or inflammatory cells may be inducing the upregulation of *MDK* and *PTN* in keloid tissue *in vivo*. A previous study found PTN to be significantly downregulated in keloid primary fibroblasts[3]; however, those authors used primary cells while we looked at whole tissue. Furthermore, they used neonatal foreskin fibroblasts as their control. This may help explain the discrepancy in results between their findings as ours.

Performing Western blots on keloid versus normal skin as well as keloid versus normal fibroblasts to determine whether MDK and PTN are upregulated at the protein level. In addition, in situ hybridization experiments for *MDK* and *PTN* on keloid sections will enable us to determine which cell type(s) are making *MDK* and *PTN* in keloid tissue.

CONCLUSION

MDK and *PTN* are increased in expression in keloid tissue compared to normal skin tissue.

The upregulation of *MDK* and *PTN* is not as robust in keloid primary fibroblasts when compared to normal skin primary fibroblasts.

REFERENCES

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