Exploring the mechanisms of keloid recurrence

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BACKGROUND

Keloids are abnormal, expanding pathological disorder that usually develop following skin trauma including injury, surgeries and burns. They are thought to be caused by an imbalance between extracellular matrix (ECM) deposition and degradation. Keloid treatment usually involves different stages and a combination of therapies. However, no standard method to treat keloids has been established, and all the current therapeutic options are accompanied with a recurrence rate that can reach up to 100% after certain types of treatment, such as surgical excision. Keloid recurrence is a serious health problem that significantly impacts patients' lives, leading to physical impairment and emotional and psychological consequences, increased healthcare costs, and repeated therapeutic interventions. As there is a lack of studies on the drivers of recurrence, this study aims to understand the molecular and cellular mechanisms behind keloid recurrence, which will be crucial to improve clinical management and patient outcomes.

METHODS

Skin tissue recurrent keloid samples were excised from two different body sites of the same patient and compared to another recurrent keloid from a different donor and two normal skin tissue samples. Single-cell RNA sequencing (scRNA-seq) and library preparation of the samples was performed following standard protocols. Raw sequencing reads were processed for quality control before normalization, clustering, annotations and integration. Differential gene expression analysis and pathway enrichment analysis were performed on each sample individually, followed by cluster-level gene expression analysis and pathway analysis for each specific cluster from different anatomical sites. Immunohistochemistry was performed to quantify collagen depositions. Finally, to investigate the role of secreted factors in keloid maintenance and recurrence, migration assays were performed with normal fibroblasts co-cultured with a mixture of basal media and conditioned media from primary and recurrent keloid fibroblasts.

RESULTS

Our initial results indicate that the cellular composition is conserved between recurrent keloid samples from different body sites, including the chest and the shoulder. These findings were supported by analysis of another recurrent keloid sample from an unrelated donor and different body site, the ankle. Differential expression analysis and pathway enrichment analysis at the cluster-level revealed conserved transcriptional profiles and enriched biological processes between all the clusters of different body sites, with the exception of a T

cell cluster, which showed significant differences in a small set of genes engaged in

vascular development, and metabolic processes, including response to reactive oxygen species and hydrogen peroxide, as well as positive regulation of nitric oxide biosynthesis. When compared to normal skin, a decreased abundance of neutrophils was observed in the disease samples. Interestingly, fibroblast and chondrocyte clusters showed an overlap in their transcriptional profile and enriched biological pathways across all samples.

To investigate whether keloid fibroblasts secrete factors that influence cellular behaviour, we performed in vitro migration assays with normal fibroblasts co-cultured with keloid conditioned media. Although not statistically significant, there was a trend towards faster migration and decreased collagen deposition in normal fibroblasts co-cultured with conditioned media derived from primary keloid compared to recurrent keloid, which aligns with the known migratory behavior of primary and recurrent keloid fibroblasts and suggests that recurrent keloids may have distinct phenotypes compared to primary keloid. We are currently repeating these experiments to develop a deeper understanding of the mechanisms underlying these differences.

CONCLUSİON

In this study we report a conserved cellular composition of recurrent keloid samples from different body sites. Differential gene expression was observed in a few gene sets and their associated biological processes in the T cell cluster originating from shoulder body site, indicative of body-site specific differences in keloid phenotype. There were differences identified in the abundance of neutrophiles in diseased samples compared to normal skin, however further investigation and larger sample sizes are needed to confirm these trends. In addition, normal fibroblasts showed a variable response to conditioned media from primary verses recurrent keloid fibroblasts, indicating distinct signalling effects associated with primary and recurrent keloid. Understanding the differences between primary and recurrent keloid and their underling mechanisms is crucial for developing better therapeutic choices and prevention strategies to reduce the burden of keloid recurrence.