Transcriptomic Profiling of Steroid Treated Keloid Fibroblasts Identifies Multiple Potential Biomarkers for Predicting Steroid Responsiveness for Keloid Therapy

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Running Title
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BACKGROUND

Intralesional corticosteroids are commonly used to treat keloids, albeit with highly variable responses. In a recent online survey of over 800 patients, Tirgan reported that only about 34% of keloid patients claimed to have benefited from steroid therapy, while 49% did not respond to it and the remaining 17% of patients actually saw a worsening of their symptoms upon steroid therapy. We reported a similar response of patient-derived keloid fibroblast proliferation in vitro upon exposure to the steroid triamcinolone acetonide (TA). This allowed us to categorize the samples as steroid sensitive if their proliferation was inhibited by steroid, and steroid resistant if their proliferation was either unaffected, or if their proliferation was accelerated (hyperproliferation) upon steroid treatment. It is likely that genetic and/or epigenetic heterogeneity between individuals determine the variable responses observed upon steroid treatment of keloids in patients or keloid fibroblasts in vitro. These genetic and/or epigenetic differences can potentially be reflected in the gene expression profiles of these keloids as determined by transcriptomic analyses. However, a comprehensive transcriptomic analysis of multiple steroid treated keloid samples is lacking, and as such, no molecular biomarkers for steroid responsiveness of keloids have emerged from the transcriptomic studies performed on keloids in the past.

METHODS

Six surgically excised keloids were obtained from local dermatology and plastic surgery clinics following Institutional Review Board (IRB) approval. Primary keloid fibroblasts were cultured from these samples and their proliferative response to steroid treatment was assessed in vitro, based on which they were assigned to steroid sensitive or resistant groups. We performed ribosomal RNA depleted total RNA sequencing (RNA-seq) based transcriptomic analysis on 3 steroid sensitive and 3 steroid resistant keloid fibroblast samples, both before and after treatment with 10μM TA for 4 days in an attempt to uncover the genes involved in their differential response to steroid treatment. In all, 36 samples were sequenced for this transcriptomic analysis (6 patient samples treated with or without steroid, each repeated 3 times). The resulting data has helped us identify several genes whose expression levels can be potentially used to predict the response of keloid patients to steroid therapy prior to initiating treatment. The expression levels of these genes were further confirmed by quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

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RESULTS

From the list of differentially expressed genes between steroid sensitive and resistant keloid fibroblasts, a total of 6 genes were identified whose expression levels correlated strongly with either sensitivity or resistance to steroids, as measured by the rate of proliferation of the keloid fibroblasts upon steroid treatment. Each of these 6 genes can potentially serve as molecular biomarkers for steroid responsiveness either independently, or in various combinations, using RT-PCR to detect mRNA corresponding to these genes. Additionally, proteins corresponding to 3 of the 6 genes identified above are known to be detectable in body fluids such as saliva and blood, suggesting that it may be possible to develop convenient non-invasive screening tests based on Enzyme-Linked Immunosorbent Assay (ELISA), or a lateral flow based rapid antibody test to determine steroid responsiveness of keloid patients prior to initiating therapy. Although the initial data was obtained from just 6 patient samples due to the significant sequencing costs involved, we are currently in the process of expanding our patient numbers and we hope to screen RNA/proteins corresponding to these potential biomarkers from saliva as well as keloid fibroblasts from at least 25 keloid patients, which will give us over 80% statistical power.

CONCLUSION

After decades of empirically determined intralesional steroid therapy for keloids that often failed, we now offer hope for the possible development of a molecular biomarker based non-invasive screening test for determining steroid responsiveness of keloid patients prior to initiating steroid therapy. In the future, such a test would greatly reduce unnecessary suffering and costs incurred by steroid resistant keloid patients who can choose alternative therapies, while increasing the compliance of the steroid sensitive keloid patients. Our results also highlight the importance of applying genomic sequencing-based approaches to study keloids as they can generate a wealth of clinically actionable information.

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