COMMON MECHANISMS AND SIGNALING PATHWAYS IN FIBROBLASTS OF CANCER-PRONE GENODERMATOSES

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Abstract

Despite the genetic bases of many rare skin diseases having been elucidated in the past years, the mutations in the causal genes often fail to explain on their own the vast array of phenotypic manifestations in these pathologies. Recessive Dystrophic Epidermolysis Bullosa (RDEB), Kindler syndrome (KS) and Xeroderma Pigmentosum C (XPC) are three genodermatoses that share a number of features, including a predisposition to cancer, whose mechanisms are not yet fully understood. In this study we have investigated the transcriptional signature across these conditions to address the role of dermal environment in the development of the pathology. Fibroblasts isolated from several RDEB, XPC and KS patients, as well as healthy donors, have been studied using RNA-Seq technology. The analysis included a thorough examination of the differentially expressed genes, a functional enrichment analysis and the determination of the affected signaling circuits using computational models of signaling pathway activity. The results revealed a set of 227 genes and 42 signaling circuits commonly altered in all three conditions, along with unique mechanisms acting in each of them. The common signaling circuits and biomarkers uncovered by the analysis point towards a phenotype in which the fibroblasts of these conditions show similarities to cancer-associated fibroblasts, enabling a favorable environment for tumor development and progression. We anticipate this approach, focused on the mechanisms that govern cell behavior, to be useful in explaining obscure phenotypic traits of these genodermatoses and to increase the number of targets for effective therapeutic intervention.

Introduction

Kindler Syndrome (KS), Recessive Dystrophic Epidermolysis Bullosa (RDEB) and Xeroderma Pigmentosum C (XPC) are three autosomal recessive skin disorders caused by mutations in FERMT1 (a component of focal adhesions in epithelial cells), COL7A1 (main component of anchoring fibrils that connect the epidermal basement membrane to the dermal tissue) and the XPC gene (involved in nucleotide excision repair pathway, the most versatile DNA-repair mechanism), respectively.

Despite the differences in the causing mutations, these genodermatoses share several traits, including cancer predisposition (all three), dermo-epidermis fragility (KS and RDEB) and photosensitivity and premature aging (KS and XPC). The goal of this project is to find common and specific molecular signatures, that can give an insight into the phenotypic characteristics of these rare diseases.

Materials and Methods

Figure 1

A) Schematic representation of RNA extraction and sequencing method.
B) Bioinformatic approach used for data analysis.
C) MDS plot of all samples.

Conclusions

The global approach proposed in this work provides the bases for the study of the common molecular mechanisms present in different rare genodermatoses. We conclude that the fibroblasts gene expression profiles share an extensive number of similarities with those of the CAF phenotype. Injury-related signals induced by the different diseases converge in an irreversible activation of the fibroblast, and an aberrant interaction between the cell and its surroundings. The final consequence is an altered stroma that resembles the one found in tumor microenvironment, and may contribute to the cancer-proneness of these conditions.

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Results: differentially expressed genes

Figure 2

A) Venn diagram showing the number of differentially expressed genes found for each of the disease vs healthy contrasts and intersections. 227 genes differentially expressed are common to the 3 genodermatoses.
B) Number of overexpressed (red), non DE (gray) and repressed (blue) shared genes between diseases.
C) logFC of the common DE genes (227). All genes are altered in the same direction in every disorder.

Results: common functional profiles

Figure 3

Most significant ontologies and KEGG pathways shared in all three comparisons Disease versus Healthy. Results can be divided in four clusters:

- Transcription factors
- Chemokine pathways
- Cancer mechanisms
- Plasma membrane & ECM

Results: signaling pathways analysis

Figure 4

A) Number of unique and shared signaling circuits defined within signaling pathways in each comparison.
B) Example of common altered signaling circuit, overexpressed in all three disorders through the BCL2 effector protein.

Results: A common CAF-like phenotype

The transcriptomic profile reveals signaling circuits which induce cells to become irreversibly activated. Antiapoptotic signals together with an altered interaction with the extracellular matrix, evidenced by the presence of previously reported and new markers (TNC, POSTN, TGFBI...), reveals a phenotype similar to that found in cancer-associated fibroblasts (CAF).

Figure 5

A) Protein-protein interaction network of common dysregulated genes includes extracellular matrix markers (cluster C) and transcription factors (clusters A & B).
B) Western blot validation, including additional patients (*), of relevant ECM protein markers.