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Research paper

Insights into protein interaction networks reveal non-receptor kinases as significant druggable targets for psoriasis

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ABSTRACT

Psoriasis is a chronic disease of the skin characterized by hyper proliferation and inflammation of the epidermis and dermal components of the skin. T-cell-dependent inflammatory process in skin governs the pathogenesis of psoriasis. An *in-silico* search strategy was utilized to identify psoriatic therapeutic drug targets. The gene expression profiling of psoriatic skin identified a total of 427 differentially expressed genes (DEGs). Gene ontology investigation of DEGs identified genes involved in calcium binding, apoptosis, keratinisation, lipid transportation and homeostasis apart from immune mediated processes. The protein interaction networks identified proteins involved in various signaling mechanisms with high degree of interconnections. The gene modules derived from the main network were enriched with rich kinome. These sub-networks were dominated by the presence of non-receptor kinase family members which are major signal transmitters in immune response. The computational approach has aided in the identification of non-receptor kinases as potential targets for psoriasis drug development.

1. Introduction

Psoriasis is a chronic inflammatory disease infecting around 2–3% of world's population (Duffin et al., 2008; http://www.psoriasis.org). The most common clinical manifestations of psoriasis includes the appearance of dry, red lesions of skin covered with silvery scales on elbow, scalp and knees (Sticherling, 2005). The disease is triggered by complex interplay of genetic and environmental factors resulting in skin barrier disruption and immune dysfunction (Chandran and Raychaudhuri, 2010). Psoriatic lesions are characterized by increased proliferation and abnormal differentiation of keratinocytes, infiltration by activated T-helper lymphocytes and neutrophils and activation of the cutaneous vasculature. These changes correspond to altered expressions of growth factors and their receptors, pro-inflammatory cytokines, and angiogenic peptides (Liu et al., 2007). The major pathophysiology of psoriasis is focused on keratinocytes and immune system. The association between psoriasis and various loci of immune system such as TH17 pathway (IL12B, IL23A, IL23R, TRAF3IP2, TYK2), innate immunity signaling pathway (NFκB, IFN, TNFAIP3, TNIP1, NFKBIA, REL, IFIH1, IL23RA), β-defensin, TH2 pathway (IL4, IL13) and adaptive immune pathway mediated by CD8 T cells (ERAP1, ZAP70) are studied by various scientific communities globally (Yao et al., 2008; Mabuchi et al., 2012).

Abbreviations: TH17, T helper 17 cell; IL12B, Interleukin 12 subunit beta; IL23A, Interleukin-23 subunit alpha; IL23R, Interleukin 23 receptor (type I cytokine receptor); TRAF3IP2, TRAF3 Interacting Protein 2; TYK2, Tyrosine kinase 2; NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; IFN, Interferon; TNFAIP3, Tumor necrosis factor, alpha-induced protein 3; TNIP1, TNFAIP3 interacting protein 1; NFKBIA, Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; REL, V-Rel Avian Reticuloendotheliosis Viral Oncogene Homolog; IFIH1, Interferon induced with helicase C domain 1; IL4, Interleukin 4; IL13, Interleukin 13; ERAP1, Endoplasmic reticulum aminopeptidase 1; ZAP70, Zeta-chain (TCR) associated protein kinase 70 kDa; CXCL1, Chemokine (C-X-C motif) ligand 1; KYNU, Kynureninase; CXCL2, Chemokine (C-X-C motif) ligand 2; S100A9, S100 calcium binding protein A9; PPARG, Peroxisome proliferators-activated receptor gamma; IL1B, Interleukin 1, beta; IL8, Interleukin 8; IL1RN, Interleukin 1 receptor antagonist; CXCL9, Chemokine (C-X-C motif) ligand 9; CCL18, Chemokine (C-C motif) ligand 18; CCL27, Chemokine (C-C motif) ligand 27; CCL20, Chemokine (C-C motif) ligand 20; PTPRC, Protein tyrosine phosphatase, receptor type, C; BCL2, B-cell CLL/lymphoma 2; LCK, Lymphocyte-specific protein tyrosine kinase; BCL3, B-cell CLL/lymphoma 3; PATZ1, POZ (BTB) and AT hook containing zinc finger 1; TP63, Tumor protein p63; LDLR, Low density lipoprotein receptor; ARSF, Arylsulfatase F; GALNT6, Polypeptide N-acetylgalactosaminyltransferase 6; TCHH, Trichohyalin; SPRR2C, Small proline-rich protein 2C; SPRR1A, Small proline-rich protein 1A; TGM1, Transglutaminase 1; TGM3, Transglutaminase 3; LYN, v-yes-1 Yamaguchi sarcoma viral related oncogene homolog; WNT5A, Wingless-type MMTV integration site family, member 5A; KLK6, Kallikrein-related peptidase 6; LPL, Lipoprotein lipase; PRR, Pattern recognition receptors; CAM, Cell-adhesion molecules; MAPK, Mitogen activated protein kinases; CSK, C-src tyrosine kinase; WNT, Wingless-type MMTV integration site family; STAT, Signal transducer and activator of transcription; SHC1, SHC (Src homology 2 domain containing) transforming protein 1; MAPK14, Mitogen-activated protein kinase 14; KRT18, Keratin 18; STAT3, Signal transducer and activator of transcription 3; STAT1, Signal transducer and activator of transcription 1; PPARGC1A, Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PRKCI, Protein Kinase C, Iota; CDH1, Cadherin 1, Type 1; EZR, Ezrin; MAPT, Microtubule-associated protein tau; FYN, FYN Oncogene; MATK, Megakaryocyte-associated tyrosine kinase; JAK1, Janus kinase 1; SRC, V-Src Avian Sarcoma (Schmidt-Ruppin A-2) Viral Oncogene Homolog; BTK, Bruton agammaglobulinemia tyrosine kinase; BMX, Bone Marrow Tyrosine Kinase Gene in Chromosome X Protein; TEC, Tec Protein Tyrosine Kinase; SYK, Spleen tyrosine kinase; MCC, Maximal clique centrality.

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The multi-factorial disease shows a strong association with arthritis, spondylotic joint disease, atherosclerosis and atopic dermatitis (Park et al., 2011). Despite intensive study over past few years the etiology and remission of psoriasis still remain obscure. The treatment regimens of psoriasis include topical treatments, phototherapy and combination therapy such as PUVA (Psoralen plus ultraviolet A therapy). Psoriasis is associated with a high degree of morbidity and the current medications can have severe side effects which include cosmetic unacceptability and significant toxicity (Mendonça and Burden, 2003).

The current medication scenario provides us with a challenge to identify new therapeutic targets that are more directly related to disease. In recent years, large numbers of disease markers are identified through genome wide expression profiles. Microarray gene expression profiling emerged as a powerful and promising tool to identify therapeutic targets in complex diseases (Spellman et al., 1998).

With increase in human protein interaction data, the network analysis provides crucial insights of molecular mechanisms of diseases (Barabási and Oltvai, 2004). Sub-networks which form the subset of large protein interaction network identify many known and novel genes from distinct interaction patterns. They provide new ways for elucidating pathways involved in disease pathogenesis.

Our work aims to ascertain various dysregulated genes and pathways involved in the pathogenesis of psoriasis and to identify novel and potential therapeutic psoriatic targets. We used gene expression profiles to create protein interaction networks in order to determine the markers of psoriasis. The topological metrics of the central network identified crucial hub proteins. The hub proteins formed the basis for deriving the sub-networks which resulted in identification of novel genes and pathways.

2. Materials and methods

2.1. Gene expression analysis

To identify the differentially expressed genes in psoriasis, we compared the expression profiles of lesional and non-lesional skin samples from 13 patients with plaque type psoriasis. The gene expression dataset GDS2518 (Reischl et al., 2007) was obtained from NCBI Gene Expression Omnibus (GEO). Expression data were generated using GCRMA (GeneChip Robust Multiarray Averaging) method implemented in affy package of R/Bioconductor (Gautier et al., 2004). Two filters were applied to minimize the dimensionality of the data and eliminate the false discovery rate (FDR). Limma package (Ritchie et al., 2015) was also used to mine the genes with a threshold of logFC > 2 and p-value (t-test) <0.05. The annotations of the probes for each gene were assembled using hgu133a.db and annotate packages of R. Each probe was matched to its corresponding gene and the probes were discarded if they did not match a gene. In case of multiple probes for a single gene, the average expression values of the probes were used as gene expression value of the candidate gene.

2.2. Gene ontology

The gene ontology analysis (Pavlidis et al., 2004) which aids in the identification of molecular function, biological process and cellular locations of large-scale transcriptome data was monitored for all the initially filtered genes using DAVID web server (Dennis et al., 2003). The hypergeometric distribution count >2 and FDR <0.05 were set as threshold criteria to identify the functional gene ontology of the mined gene set.

2.3. Cellular pathways and protein–protein interactions

Molecular signature database (MSigDB) (Liberzon et al., 2011) was used to obtain the biological pathways for the initially screened dataset (Ponzoni et al., 2014). We chose canonical pathways which include pathways from BioCarta, KEGG and Reactome databases.

2.4. Protein–protein interaction network

The human protein–protein interactions (PPIs) were downloaded from Human Protein Reference Database (HPRD), as of March 2014 (Peri et al., 2004). The interactions in HPRD were obtained using in-vivo, in-vitro and yeast 2-hybrid assays. The retrieved HPRD data was parsed using Perl scripts. The protein–protein interaction (PPI) networks were constructed and visualized using Cytoscape 3.2.0 (Shannon et al., 2003). Topological properties (Jonsson and Bates, 2006) of the PPIs characterize protein functionality and molecular mechanisms of diseases. Hence we computed the topological metrics like degree of distribution of proteins, average number of neighbors, network density and clustering co-efficient using Cytoscape's Network Analyzer plugin. Hub proteins interact with large number of partners and often play crucial roles by maintaining the cellular control. The top 15 hub proteins were narrowed down by selecting frequently appearing proteins classified by Hubba plugin based on Maximal clique centrality (MCC), closeness, degree and betweenness measures.

2.5. Clustering and subnetworks

The highly interconnected hub proteins filtered from the main network were considered as central node for clustering using MCODE plugin. The MCODE with a node cut-off of 0.2, K-Core of 2, fluff-density cut-off of 0.2 and max-depth of 100 was initialized. The sub-networks were then validated by subjecting them to Gene ontology identification and gene-functional clustering using DAVID server.

3. Results and discussion

3.1. Identification of differential expressed genes from transcriptome

The comparison of 13 lesion and non-lesion skin samples of psoriasis patients resulted in identification of 244 up-regulated genes and 183 down-regulated genes (S.Table 1). From these differentially expressed genes, we noticed many genes reported to be associated with psoriasis. The ontology of the filtered genes was investigated and presented in Fig. 1. Genes up-regulated in psoriatic lesions compared to non-lesional psoriatic skin were enriched in intracellular signaling, defense response, homeostatic process, response to wounding, regulation of apoptosis, T cell activation, cell proliferation, protein kinase cascade, cytokine mediated signaling and leukocyte activation, while genes down-regulated in the lesional skin were enriched with cell adhesion, response to organic substance, hormone stimulus and endogenous stimulus, cytoskeleton organization, blood circulation, lipid biosynthetic process and regulation of growth. However, the top leads for overall gene patterns were involved in processes such as immune response, intracellular signaling cascade, cell adhesion, cytoskeleton organization, regulation of cell proliferation and apoptosis.

3.1.1. Differentially expressed gene involvement in immune response intracellular signaling cascade

We noted that majority of the genes in the immune response and intracellular signaling cascade were up-regulated. There is a significant interest in the role of genes involved in intracellular signaling cascades of inflammation. Transmigration of circulating leukocytes across the endothelium is one of the key processes of inflammation. Neutrophils, the active participants of inflammatory reactions accumulate in the epidermis marking a histological characteristic of psoriasis. Various mediators implicated in neutrophil chemotaxis are expressed more in the immune response and signaling cascade category. For instance genes involved in neutrophil attraction such as CXCR2, CXCR4 and ligands CXCL1 and CXCL2 (Nickoloff et al., 2007). CXCL8 (IL8) a potent chemoattractant