Original Article

Modeling signaling pathways leading to wrinkle formation: Identification of the skin aging target

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ABSTRACT

Background: In the present scenario, wrinkle formation, prominent sign of skin ageing, is one of the most demanding areas of research. This burgeoning research demand to reduce, delay and restore the effects of skin ageing has led to the study of various signaling pathways leading to wrinkle formation. Wrinkles appear on skin due to influence of intrinsic and extrinsic factors on mitogenic reactions and signal transduction pathways. **Aim:** The aim of the present study is to analyze each protein involved in the signaling pathway leading to dilapidation of collagen and an attempt has been made to compare different signal transduction pathways to identify a common target for skin ageing. **Methods:** In the present work, bioinformatics tools have been used to extract information from already existing experimental data. The statistical techniques are used for further analysis and make useful predictions for skin ageing. **Results:** Stressors like UV irradiation, osmotic stress and heat shock have been reported to activate epidermal growth factor receptor, interleukin 1 receptor, tumor necrosis factor receptor, platelet-derived growth factor receptor and platelet activation factor receptor signaling pathways, which lead to the production of matrix metalloproteinases, collagen degradation and, consequently, wrinkle formation. When all the five signaling pathways were modeled, the c-jun part of the AP-1 transcription factor was found to be a common intermediate protein involved in all the signaling cascades. Moreover, it shows differential expression in the skin on response to stressors. **Conclusion:** We proposed c-jun to be the most potent target for drug designing against wrinkle formation.

Key words: Ageing target, Distance matrix, Signaling pathways, Skin ageing, Stressors

INTRODUCTION

Skin ageing is one of the most demanding areas of research, as now-a-days it is more significant to prolong health expectancy, allowing people to look younger and live healthy lives rather than to prolong life expectancy. Skin ageing is characterized by signs like discoloration, wrinkles and texture loss. The two major types of skin ageing are intrinsic ageing and extrinsic ageing. Intrinsic ageing is genetically programmed ageing, also called chronological ageing, whereas extrinsic ageing is due to environmental factors like sunlight, mainly UV rays (commonly known as photoageing), stress and pollution.[1-2] These factors activate the receptors of signal transduction pathways through a ligand-independent mechanism.[3] Chronological ageing and photoageing have different signs and they differ also in the electron microscopic appearance but no longer considered separate entities as they share some important molecular features.[4-5] Consequently, it can be concluded that extrinsic ageing is superimposition on intrinsic ageing and is thus called premature ageing.

One of the most prominent signs of skin ageing is wrinkle formation. Wrinkles appear on the skin due to loss of elasticity caused by rapid degradation of collagen. This deficiency is contributed by the influence of intrinsic and extrinsic factors on mitogenic response and signal transduction pathways. Receptors like epidermal growth factor receptor (EGFr), interleukin-1 receptor (IL-1r), tumor necrosis factor receptor (TNFr), platelet-derived growth factor receptor (PDGFr) and platelet-activating factor receptor (PAF-r) on activation

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stimulate tyrosine kinase and the associated adapter proteins, which transfer the signal to the transcription factor activation protein 1 (AP-1) and initiate the production of matrix metalloproteinases (MMPs).[6] This disturbs the natural balance between MMPs and mmp inhibitors, resulting in loss of collagen in the skin.[7] AP-1 transcription factor is composed of two subunits: c-fos and c-jun. In human skin, AP-1 activity is limited by c-jun expression because c-fos is continuously expressed. Interestingly, while c-fos expression in young (18–28 years old) and aged (80 years old) skin does not differ, c-jun expression is elevated in aged compared with young skin.[8]

In order to reduce, delay and restore the effects of skin ageing, it is highly important to completely understand its biological process. The present study is proposed to analyze each protein involved in the pathway leading to dilapidation of collagen and an attempt has been made to compare different signal transduction pathways to identify a common target for both types of ageing so that when targeted through drug design, the intensity of collagen degradation will decline. In the present work, bioinformatics tools have been used to extract information for already existing experimental data. The statistical techniques are used for further analysis and make useful predictions for skin ageing.

METHODS

On the way to unearth the proposed findings, the literature was thoroughly studied to identify the proteins involved in the signal transduction pathway leading to wrinkle formation. Further, protein–protein interaction between these identified proteins was derived using String.

String (http://string.embl.de) is a database of interacting proteins and a tool for protein networking. String provided search of the protein interactant and gave a diagrammatic representation of these interactants in the form of a protein network. It is a rich database of interacting proteins collected from experiments, text mining, databases, coexpression, cooccurrence, gene fusion, neighbor proteins and homology proteins. However, for the present work, only experimentally validated protein interactants (joined together by violet lines) were considered to construct the signal transduction pathway.

Sometimes, a single protein has more than one interactant in the same pathway, which may create a problem in finding the accurate signaling pathway. To solve the problem of finding the exact flow of signals between various interactants in a signal transduction pathway, Pearson correlation coefficient (r) was calculated. More the value of the Pearson correlation coefficient, more closely the proteins are evolutionarily related to each other. In this method, coevolutionary information of the interacting partners was gathered and correlation was applied between the distance matrices, where each matrix stores the pairwise distances between a protein and its orthologues from a group of reference genomes.[9] To calculate the Pearson correlation coefficient, orthologues of each interacting protein were collected from the Kegg/Ko database. Then, a distance matrix was generated from the protein and its orthologous sequences by the ClustalW software. ClustalW software is used for multiple sequence alignment. Once a distance matrix is obtained, it is converted to a phylogenetic vector by taking the lower half of the elements of the distance matrix. Then, normalization of the phylogenetic vector is carried out by the following formula:

$$\text{Normalized vector} = \frac{\text{Element of phylogenetic vector} - \text{mean of all the elements of phylogeneic vector}}{\text{Standard Deviation of all the elements of phylogenetic vector}}$$

As discussed above, normalized vectors were constructed for all the interacting proteins. From these normalized vectors, the Pearson correlation coefficient was calculated between the desired proteins.

The method discussed above for calculation of the Pearson correlation coefficient is illustrated by taking an example of the EGFr protein sequence (of homosapiens).[10] We have collected orthologues of EGFr from 10 different eukaryotes (like rat, dog, pig, cow, opossum, chicken, zebrafish, chimpanzee, mouse and monkey). Then, all these 11 sequences were subjected to ClustalW to obtain a distance matrix, as shown in Figure 1. Figure 1 shows a symmetric matrix. The lower half of the matrix was used to generate the phylogenetic vector. With 11 protein sequences, 55 elements were generated in each phylogenetic vector according to the formula $\{(n^2-n)/2\}$. Now, the phylogenetic vector obtained was normalized by the above formula and the Pearson correlation coefficient was calculated between EGFr and other protein interactants.

HSA	0.000	0.504	0.914	0.213	0.098	0.945	0.945	0.917	0.931	0.454	0.931
RNO	0.504	0.000	0.931	0.432	0.498	0.943	0.940	0.931	0.936	0.509	0.942
CFA	0.314	0.931	0.000	0.913	0.911	0.951	0.948	0.950	0.948	0.933	0.942
SSC	0.213	0.432	0.913	0.000	0.204	0.936	0.935	0.925	0.936	0.509	0.940
MCC	0.098	0.498	0.911	0.204	0.000	0.924	0.941	0.919	0.923	0.387	0.934
BTA	0.945	0.943	0.951	0.936	0.924	0.000	0.941	0.932	0.932	0.926	0.928
MDO	0.945	0.940	0.948	0.935	0.941	0.941	0.000	0.934	0.942	0.945	0.942
GGA	0.917	0.931	0.950	0.925	0.919	0.932	0.934	0.000	0.943	0.934	0.924
DRE	0.931	0.936	0.948	0.936	0.923	0.932	0.942	0.943	0.000	0.925	0.892
PTR	0.454	0.509	0.933	0.509	0.387	0.926	0.945	0.934	0.925	0.000	0.936
MMU	0.931	0.942	0.942	0.940	0.934	0.928	0.942	0.924	0.892	0.936	0.000

Figure 1: Distance matrix obtained from EGFr protein sequences

RESULTS

Altogether, there were 30 proteins studied from the literature that were found to be involved in collagen degradation. After deriving the protein interaction between the identified proteins and calculation of the Pearson correlation coefficient between the proteins that have more than one interactant, signaling pathway leading to wrinkle formation was modeled.

EGFr and associated proteins involved in the signaling pathway

According to the literature, UV-activated EGFr is at least partly responsible for the activation of extracellular signal-regulated kinases (Erk1/2) directly, which then activates c-jun.[11,12]

String determined growth factor receptor-bound protein 2 (Grb2), Ras GTPase-activating protein 1 (Rasa1), mitogen-activated protein kinase 1 (Mapk1/Erk2) and mitogen-activated protein kinase 3 (Mapk3/Erk1) to be the interactants of EGFr. To determine the strength of the interaction of these proteins with EGFr, the Pearson correlation coefficient was calculated [Table 1 A]. Because EGFr-Mapk1 showed the maximum strength of interaction, it can be concluded that EGFr directly activates Mapk1, i.e. Erk2 rather than Erk1, which further transfers the signal to c-jun [Figure 2 A]. Moreover, EGFr-Mapk3 is not an experimentally proved interaction.

PDGFr and associated proteins involved in the signaling pathway

PDGFr also leads to the production of MMPs via activation of Erk 1/2 and c-jun on stress. In vascular smooth muscles cells, factors ranging from physical exertion to psychological stress activate intracellular signaling and stimulate the synthesis of alfa platelet-derived growth factor receptor (PDGFrA) and transcription factor c-fos/c-jun.[13] In fibroblast cells, beta platelet-derived growth factor receptor (PDGFrB) activates c-jun via the Erk1/2 pathway.[14] These are ligand-dependant activation of receptors. However, UV rays activate the PDGFrB pathways, leading to MMPs production, which is ligand-independent activation.

String confirmed that PDGFrB and PDGFrA interact with Grb2 experimentally. Grb2 further interacts with Mapk1 and Mapk3 and transfers the signal to c-jun. In order to determine the most potent interactant among them, the r-value was calculated [Table 1B]. Grb2-Mapk1 showed the maximum r-value and therefore it was considered as an intermediate protein involved in the pathway. Moreover, Grb2-Mapk9 interaction was not experimentally proved. The concluded signaling pathway of PDGFr is shown in Figure 2E.

PAF-r and associated proteins involved in the signaling pathway

According to the literature, UV light irradiation and acute thermal damage activates PAF-r-mediated activation of Erk1/2. PAF-r, not directly but indirectly, brings about activation of Erk1/2. The transactivation of EGFr by PAF-r involves a matrix metalloproteinase-dependent cleavage and secretion of heparin-binding EGF-like growth factor (Hb-Egf) that activates the EGFr and subsequently phosphorylates Erk.[15]

String fully supports the literature and showed an interaction between Hb-Egf and EGFr and then EGFr interacting with Mapk1 (potent interactant than Mapk3), carrying the signal to c-jun [Figure 2B].

TNFr and associated proteins involved in the signaling pathway

TNFr also participates in the production of MMPs when

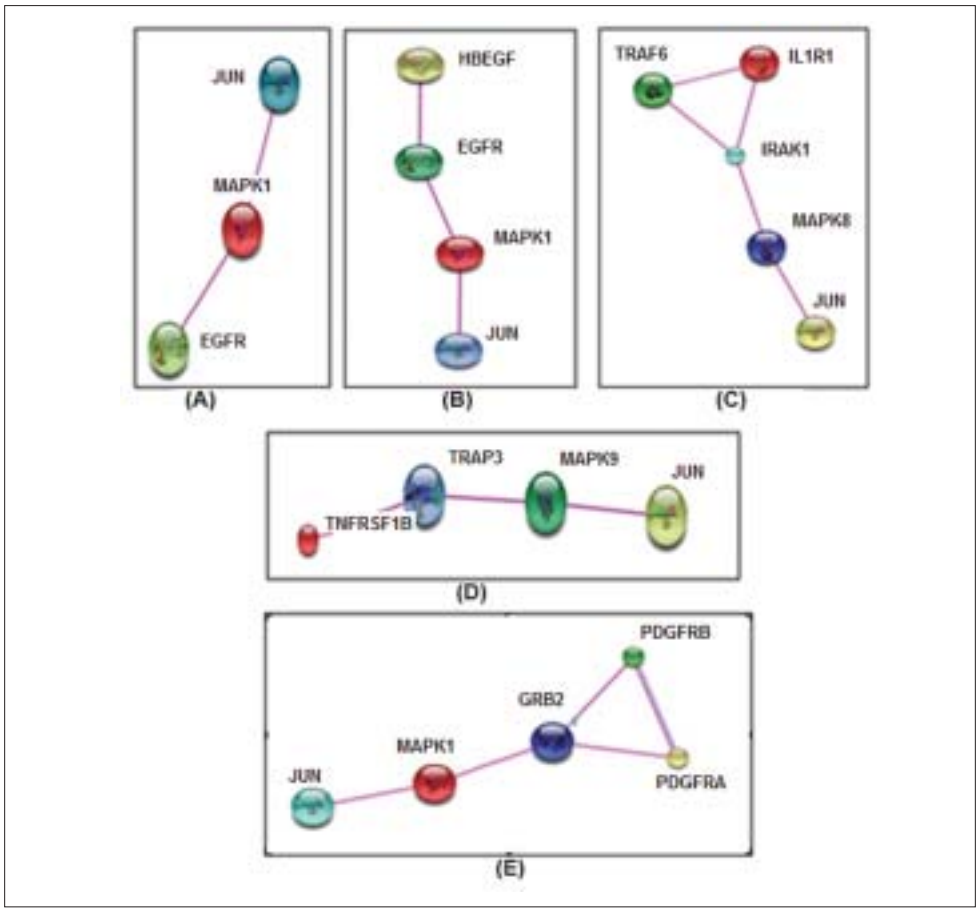

Figure 2: (A) EGFr-mediated, (B) PAF-r-mediated, (C) IL-1r1-mediated, (D) TNFr–mediated and (E) PDGFr-mediated signaling pathways obtained by String

activated by environmental stress, such as UV rays. Experiments have proved that TNF receptor-associated factor 2 (Traf2/Trap3) bifurcates the TNF-mediated NF-kB and c-jun pathways. Traf2 is an adaptor protein of the tumor necrosis factor receptor superfamily member 1B (TNFr2) but not the tumor necrosis factor receptor superfamily member 1A (TNFr1). Therefore, it can be concluded that the signaling transduction pathway starts on activation of TNFr2, which further activates Traf2 and, sequentially, stress-activated protein kinase 2 (Jnk2/Mapk9) and c-jun, which leads to the production of MMPs.[16]

Table 1: List of protein interactants with their corresponding r-value involved in (A) EGFR-mediated, (B) PDGFr-mediated and (C) 1L-1r1-mediated signaling pathways

Protein interactants	r value
EGFR mediated signaling pathway (A)	
EGFR-GRB (experimentally proved)	0.180271
EGFR-RASA1 (experimentally proved)	-0.10532
E G F R.-MA P K1 (experimentally proved)	0.510844
EGFR-MAPK3 (proved by textmining)	0.066898
PDGFr mediated signaling pathway (B)	
GRB2-MAPK 1 (experimentally proved)	0.222563
GRB2-MAPK3 (proved by textmining)	0.040343
IL1R1 mediated signaling pathway (C)	
IRAK 1-MAPK8 (experimentally proved)	0.307616
IRAK 1-MAPK9 (proved by textmining)	0.273023

String, as such, generated results that have been given in the literature, i.e. TNFrsf1B combined with Trap3, which further combined with Mapk9. TNFrsf1B- Trap3, Trap3- Mapk9 and Mapk9-c-jun are experimentally proved interactants. Because Trap3 does not interact with Mapk8 experimentally, therefore, Mapk9 was considered to be involved in the signaling pathway. The concluded pathway is given in Figure 2D.

IL-1r and associated proteins involved in the signaling pathway

According to research papers, the IL-1r-mediated signaling pathway is activated by environmental stress. On activation, it activates the toll-like receptor protein interleukin-1 receptor-associated kinase-2 (Irak2), which further activates the TNF receptor-associated factor 6 (Traf6). Traf6 is a member of the

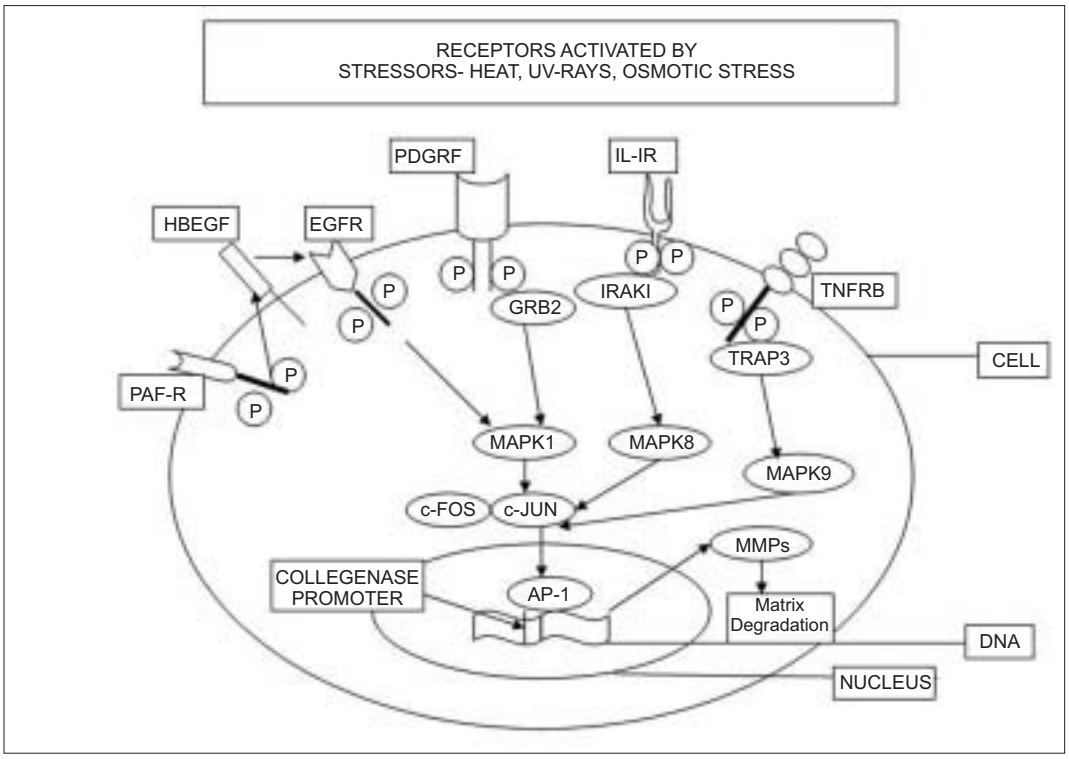

Figure 3: Model of the signaling cascade leading to collagen degradation in the skin

tumor necrosis factor receptor-associated factor family of proteins. It does not interact with Tnf. Instead, it is associated with IL-1 via Irak. After Traf6 activation, mitogen-activated protein kinase is activated, which further activates c-jun and ultimately activates MMPs, which leads to collagen degradation.[17] Iraf bifurcates the IL-1-induced NF-kB and Jnk pathways.

The String database was used for finding protein interactants involved in the signaling pathway. A list of the interactants involved in the pathway is given in Table 2.

Experimentally, IL-1r1 was found to interact with both Irak2 and Traf6. Now, according to the literature, Traf6 further carries the signal to a protein kinase, which was found to be mitogen-activated protein kinase kinase kinase 14 or NF-kappa beta-inducing kinase (Map3k14). But Map3k14 is not an interactant of c-jun. However, it activates Nf-kb2 and participates in inflammation. Moreover, Irak2 does not interact with Jnk1/2 experimentally. But, when studies with Interleukin-1 receptor-associated kinase 1 (Irak1) were performed, it was found to be an interactant of IL-1r1, Traf6 and stress-activated protein kinase 1 (Jnk1/Mapk8). Mapk8 further activates c-jun, which leads to MMPs activation. Therefore, it is proposed that Irak1 and not Irak2 bifurcates the IL-1-induced Nf-kb and Jnk pathways.

It was found that Jnk1/2 interacts with Irak1 for transferring the signal to c-jun. The Pearson correlation coefficient calculated for these proteins is given in Table 1C. Because the strength of the interaction is more for Irak1-Mapk8, it is likely to transfer the signal to c-jun. Moreover, Irak1-Mapk9 is not an experimental interactant. The concluded pathway is shown in Figure 2C.

Table 2: List of protein interactants involved in the IL-1-mediated signaling pathway

Proteins interactants
IL1R1-IRAK2
IL1R1-IRAK1
IL1R1-TRAF6
IRAK1-TRAF6
IRAK2-TRAF6
TRAF6-MAP3K14
MAP3K14-NFKB2
IRAK1-MAPK8
MAPK8-JUN
IRAK2-MAPK8
IRAK2-MAPK9

DISCUSSION

EGFr, PDGFr, PAF-r, TNFr and IL-1r1, activated by stressors like heat, osmotic stress and UV-rays, superimpose the chronological ageing and intensify the degradation of collagen and, consequently, lead to wrinkle formation. In the EGFr signaling pathway, EGFR carries signal directly to Mapk1 and activates c-jun for the production of MMPs. In the PDGFr pathway, PDGFra and PDGFrb activate Grb2, Mapk1 and c-jun for MMPs production. In the PAF-r pathway, PAF-r transactivates EGFr via Hb-Egf, which activates Mapk1 and c-jun. In the TNFr-mediated signaling pathway, TNFrsf1B carries the signal to Trap3, which further activates c-jun via Mapk9. IL-1r1 activates c-jun via Irak1. The diagrammatic representation of all these signaling pathways is shown in Figure 3.

When all the pathways were studied for a common target, c-jun was the only protein involved in all the pathways. Therefore, it is proposed to be the most potent target for drug design. Targeting this protein will decrease the degradation of collagen in the skin by blocking all these five pathways. Moreover, its expression increases in photoaged and old skin, proving its involvement in collagen degradation and wrinkle formation. Whereas expression of c-fos remains unaffected and studies indicate that c-fos-regulated genes exert a protective function, its deficiency makes the cells hypersensitive to a broad spectrum of DNA-damaging agents.[18] Therefore, it would be more effective to target c-jun instead of targeting the whole AP-1 protein (c-fos + c-jun).

At present, there is no drug for c-jun. Because we have identified the target, our next step is toward the *in silico* designing of ligands for c-jun, which, after wet lab experimentation, can be a potent drug.

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