Molecular Expression Profiles of Extracellular Matrix Proteins in Human Skin Diseases

The goal of this study was to establish a comparative expression analysis of RNA extracted from normal and diseased skin fibroblasts. Total RNA was extracted from diseased skin fibroblast cell lines and normal skin fibroblast cell lines. It was then labeled and hybridized to a commercial gene array containing genes for cell adhesion molecules and proteins of the extracellular matrix. The functional gene groupings of the arrays are categorized as cell membrane adhesion molecules, extracellular matrix proteins, proteases, and protease inhibitors. Reverse transcription polymerase chain reaction (RT-PCR) was performed to validate the findings of the gene array. A molecular profile was developed, and three candidate genes that may play a role in skin disease were identified: integrin alpha 4, integrin alpha 8, and integrin alpha 9. This study has identified differential gene expression between normal skin and different forms of skin diseases. These differential gene expression profiles may be useful in future diagnostic schemes for skin cancer and other skin diseases.

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Introduction

The extracellular environment plays an essential role in cellular behavior. Integrins are a family of transmembrane glycoprotein receptors that mediate cell-matrix and cell-cell interactions; they mediate the anchorage and migration of cells via recognition of variable extracellular matrix molecules. Intracellular signals generated by integrins often influence gene expression, affecting the regulation of cell survival, differentiation, and proliferation. In that respect, integrins are also involved in many disease processes, such as inflammation and tumor progression. In human cancer, integrins are the main link between a cell and the extracellular matrix (ECM).

Materials and Methods

The cell lines CRL-1947, CRL-1974, HTB65, CRL-7425, CRL-1762, CRL-1396, CRL-7426, and CRL-1360 were purchased from American Type Culture Collection (Rockville, Md). Cells were propagated in the recommended media and given new media every two to three days for two to three weeks.

RNA was extracted by using the TriZol Reagent (Invitrogen, Carlsbad, Calif). Cells were lysed by adding 7.5 mL TriZol directly to the flasks. Next, 1.4 mL chloroform was added and shaken for 15 seconds and then centrifuged for 10 minutes at 12,000 rpm. The aqueous phase was then transferred to a clean tube, and 3.5 mL of isopropyl alcohol was added. It was then incubated at room temperature for 10 minutes and then centrifuged at 12,000 rpm for 10 minutes. Next, it was washed with 7.5 mL 75% ethanol and centrifuged at 7,500 rpm for five minutes. The pellet was then resuspended in 150 μL RNA storage solution and stored at −80°C.

Extracellular matrix and adhesion molecules were obtained from SuperArray. Array membranes were prehybridized with denatured salmon sperm DNA in GEA hybridized solution at 60°C for two hours. For each RNA sample, the LPR cocktail (18 μL Buffer L, 9 μL Buffer AF, 2 μL Biotin-16-dUTP, and 1 μL DNA polymerase) was prepared. For each array, 30 μL of the LPR cocktail was added to each RT Reaction and mixed with a pipettor. The mixture then underwent 30 cycles of 85°C for five minutes then 72°C for five minutes. The mixture was denatured at 94°C for two minutes. The labeled cDNA was added to the membrane and allowed to hybridize overnight. The membranes were washed with 2xl SSC/.1% SDS and .1x SSC/.5% SDS and blocked with blocking solution; the probes were detected by using AP-Strepavadin, specific buffers, CDP Star, and subsequent exposure to x-ray film for 30 seconds to 45 minutes. The audiogram was analyzed by using scanalyzer and GEArray Analyzer.

To prepare each RNA sample for RT-PCR, a master mix containing 17 μL water, 5 μL buffer, .5 μL dNTP, .5 μL specific primer, 1 μL $MgSO_4$, .5 μl AMV, and .5 μl of Tfl. We added .25 μg extracted RNA to the master mix in an RT-PCR tube. The tube was then placed in a thermocycler and set to the appropriate reverse transcription polymerase chain reaction (RT-PCR) program. After the completion of the RT-PCR, the samples were then run

through gel electrophoresis on 2% agarose gels. Gels were then analyzed with an imager, which was also used to record the density of each band.

RESULTS

Integrin Alpha (ITGA) 4

In comparison to normal skin RNA (CRL 1947), ITGA4 was downregulated in metastatic melanoma (HTB65), primary in situ melanoma (CRL 1974 and CRL 7425), keloids (CRL 1762), and cutis laxa (CRL 1396). ITGA4 presence was upregulated in metastatic melanoma(CRL 7426) and xeroderma pigmentosum (CRL 1360).

ITGA8

In comparison to the normal skin RNA (CRL 1947), ITGA8 was downregulated in metastatic melanoma (HTB65 and CRL 7426), primary in situ melanoma (CRL 1974), and xeroderma pigmentosum (CRL 1360). ITGA8 was upregulated in primary in situ melanoma (CRL 7425), keloids (CRL 1762), and cutis laxa (CRL 1396).

ITGA9

In comparison to the normal skin RNA (CRL 1947), ITGA9 was only upregulated in metastatic melanoma (HTB65) and primary in situ melanoma (CRL 1974). The other skin diseases showed no changes as compared to the normal.

DISCUSSION

Integrins are receptor proteins that allow cells to bind and respond to the extracellular matrix. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions.[1] Extracellular matrix (ECM) and cell-cell interactions are important in disease processes including inflammation and metastasis; many of these interactions are directed by integrins.

The downregulation of an integrin does not indicate a single outcome; it could mean a variety of things. Downregulation could mean that the integrin supports a normal phenotype. Conversely, downregulation can mean that the integrin supports the malignant phenotype by not interfering with its proliferation. A decrease in integrin expression may even promote cell migration because maximum cell migration speed is dependent on optimal ligand concentration, integrin expression, and ligand-integrin affinity.[2] Integrins also play a role in signaling cells. In tumors, cellular instability can lead to abnormalities in the signaling molecules, leading to a possible change in integrin function.[3]

In this study, the significant downregulation of ITGA4 in primary in situ melanoma, keloids, and cutis laxa does not provide a definite answer to its role in the diseases. ITGA4 may be suppressed by these diseases or may not interfere. The upregulation of ITGA4 in xeroderma pigmentosum, however, may be a positive biomarker of the disease.

The downregulation of ITGA8 in metastatic melanoma and xeroderma pigmentosum leaves more to be explored about the specific function of the integrin in these diseases. ITGA8 may be a positive biomarker in keloids and cutis laxa. Since ITGA8 is influential in the healing of wounds, an excess of it in keloids is understandable since keloids form during the healing process. ITGA9 showed upregulation in metastatic melanoma and primary in situ melanoma, but the levels of this integrin did not change in any of the other diseases.

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