Biomarkers of Keloid Formation: Clinical Applications in Oculoplastic Surge

Maximilian R. Padilla1*, Sara Bijan2, Hershel Patel1

1Department Of Ophthalmology & Visual Sciences, University of Utah 65 Mario Capecchi Drive, Salt Lake City, UT, USA.
2Medical Student, University of South Florida College of Medicine.

Abstract
Keloids and hypertrophic scarring can turn even the most well thought out and precisely performed facial surgery into a postoperative disaster. Given that these scars can cause significant disfigurement, it would be ideal to have a biomarker that could be tested to see if a candidate for elective surgery is at risk of developing keloids. After extensive literature review we found that there are several biomarkers that can be screened for to risk stratify a patient’s likelihood of developing keloids. Some of these biomarkers that are especially telling in regards to patients risks are high levels/ increased activity of rds to patients risks are the HLA-DRB1 gene, SMAD transcription factors, TGF-β, and specific alleles within the major histocompatibility complex (MHC) family. The HLA-DRB1 gene, SMAD transcription factors, TGF-β, and specific alleles within the major histocompatibility complex (MHC) family. While it might not always be practical or cost effective to screen patients for their risk of developing keloid scar in the planning stages of elective surgery, it can certainly help to prevent poor outcomes.

Key words: Keloid; Biomarkers; Oculoplastic surgery; Facial surgery; Scarring.

Abbreviations
MMPs: Matrix Metallo Proteinase; PAI: Plasminogen Activator Inhibitors; CGRP: Calcitonin Gene-Related Peptide; SP: Substance P; MHC: Major Histocompatibility Complex; IGF-1: Insulin-Like Growth Factor-1.

Introduction
Keloid is a term derived from the Greek word chele, which translates into crab claw, because of the crab claw like appearance of the scars. Keloid scars form from an aberrant expansion of connective tissue that goes beyond the actual boundaries of the wound. Race has been shown to be a factor important factor in keloid scar formation, so much that populations at risk (African Americans and Asians) will readily reveal their predisposition during pre-operative counseling compared to their lesser-affected genetic heritages.

A distinct property of keloid scars is that they can begin to form as long as 6-8 weeks after the causative injury or surgery. This unpredictable nature of keloid scar can turn even the most well thought through and perfectly performed facial surgery into a postoperative disaster. One large sample size study showed that the majority of patients with keloids experience significant psychological and physical impairment [2].

Many factors go into the complicated process of keloid formation including: inflammation, protein formation, and immunoregulators, all of which are influenced in part by certain biomarkers that control these processes at a biochemical level. Although the exact incidence and prevalence of keloids has not been determined, it is reported that the prevalence can be as high as 5 to 16 percent of patients of Hispanic/Latin and African heritage [11]. As mentioned before, the prevalence of keloids can be attributed to racial background, leading to the conclusion that these patients share a common predisposing factor to their formation.

In patients of Northern European and Asian descent, biomarker testing for pre-operative keloid risk stratification can be a useful tool before surgery. The most significant predictor in the development of keloids is if the patient has had a prior history of this type of scarring. Patients prone to keloids, even on the extremities or trunk, are at a much higher risk during facial surgery.

There are many different therapies used to both prevent the initiation of, stop the growth of, or cause regression of keloid scars but few are entirely successful. These therapies primarily focus on direct inhibition of specific keloid forming events such as cytokine elaboration, fibroblast proliferation and collagen deposition [9]. A potential successful treatment could improve the outcome of all reconstructive surgeries, and identification of as many biomarkers could pinpoint treatment strategies.

The goal of this review is to summarize the known biomarkers that are associated with keloid formation to see if a practical biomarker can be reliably tested for before patients with questionable risks of keloid formation undergo elective facial surgery.
Biomarkers

HLA-DRB1*15 is a gene that is a part of the human leucoyte antigens (HLA) system. One study showed that the frequency of keloid development in a Caucasian population with this gene was higher than that of the control population, with almost 39% of the HLA-DRB1*15 gene carriers being affected by keloid formation [3]. This gene can serve as a valuable biomarker in predicting whether or not Caucasians of northern European descent are at risk of keloid development.

A2B1 integrin is a receptor that is found on various cell types, mainly platelets and epithelial cells. It binds collagen and laminin. When injury occurs, this biomarker is involved in platelet adhesion once the collagen fiber surface is exposed. Collagen type 1 preferentially binds to α2B1 integrin, which can result in increased production of collagen [4]. Integrin α1β1 is another related biomarker which is found on mesenchymal cells. The α1 subunit seems to be related to collagen regulation, as deficiencies and mutations in this section of the receptor have been shown to increase collagen concentration within fibroblasts in keloids. It has been theorized that people with α1 abnormalities are more prone to keloids and various types of hypertrophic scars.

Mmps (Matrix metalloproteinase’s) are involved in the breakdown and reformation of the extracellular matrix, which is important in that the synthesis and breakdown of collagen exists in a balance and any disturbance in this balance can lead to a keloid formation.

One of these key molecules is MMP-9 which is responsible for the breakdown of type 4 and 5 collagen. MMP-2 on the other hand is also significant in extracellular matrix regulation in that it breaks down type 4 collagen and in turn degrades the extracellular matrix along with cellular basement membranes [3].

Hsp70 is a protein produced when a cell undergoes stress, such as oxidative damage from heavy metals and injury. This heat shock protein has also been shown to stop apoptosis and has therefore been implicated in certain cancers. Because of its role in cell survival, there have been studies showing increased levels in keloid fibroblasts. Cells that make up keloids resist normal cell death and have unregulated proliferation much like tumor cells. When there is trauma/stress from surgery Hsp70 up regulates resulting in increased scar tissue formation. A further layer of complexity lies in the fact that Hsp70 and TGF-β levels are closely related as up regulation of Hsp70 activates TGF-β, which is involved in fibrosis, angiogenesis, and extracellular matrix production all of the factors that go into the formation of keloid [7].

PDGF is a growth factor, found within mesenchymal cells, including smooth muscle and fibroblasts, which irresponsive for many cellular functions, the most important being cellular proliferation and cellular adhesion. When PDGF binds to its receptor in the context of injury, it induces the spread of fibroblasts possibly through allowing the fibroblasts to divide rapidly by skipping stages within the cell cycle [1]. PDGF has positive effects on the formation of extracellular matrix, allowing fibroblasts and other relevant cells to migrate and assist in wound healing. Due to this an increased activity of PDGF can be used as a biomarker risk factor for keloid.

PAI-1 and PAI-2 (plasminogen activator inhibitors 1,2) are biomarkers that are involved in cell migration, adhesion, and angiogenesis. They are involved in the regulation of urokinase plasminogen activator, which synthesizes plasmin from plasminogen. This process is responsible for extracellular membrane breakdown. Organ fibrosis has been linked to high levels of PAI-1 due to its inhibitory effect on plasminogen, which in turn leads to accumulation of fibrin, a key molecule involved in scar formation [12].

CGRP (calcitonin gene-related peptide) is found primarily in the skin and fibroblasts. CGRP allows inflammatory cells such as neutrophils to migrate during times of cell injury and resulting healing process. It has also been linked to the proliferation of keratinocytes [5]. This biomarker binds to receptors on dermal fibroblasts and may be responsible for the synthesis of extracellular matrix. CGRP is also found in nerve endings and is a strong vasodilator and might cause degranulation of mast cells [5]. All of these factors make this another biomarker of keloid.

Substance P (SP) is present in large amounts in human skin, second in quantity to CGRP. It has been shown to influence human skin fibroblast proliferation along with keratinocytes and increase the growth of smooth muscle and endothelial cells [5]. SP is also an important neuropeptide involved in the wound healing process, due to its role in pain, vasodilation, and inflammation.

TGF-β, a key mediator in keloid formation, interacts closely with SMAD complexes, transcription factors that have been shown to be elevated in skin cultured from keloids. Inhibiting this interaction could possibly stop the formation of scars, and can act as a biomarker for keloid [10].

Alleles within the major histocompatibility complex (MHC) family have been shown to be involved in keloid formation. These genes include DQB1*0501. DQB was found to be elevated in Asian patients who were prone to developing keloids [3].

IGF-1 (Insulin-Like Growth Factor-1) is a biomarker that acts a hormone. Its activity is enhanced by growth hormone and therefore allows for the growth of virtually every cell of the body. This effect would possibly allow keloid formation due to increased cell deposition and uncontrolled cell division. Fibroblasts that had high levels of IGF-1 and resulting protein mrna were found to divide without requiring any additional external factors and resisted undergoing apoptosis, along with fibroblast invasion [13]. IGF-1 is also involved in growth of scar tissue. Collagen production and fibroblast invasion were found to be inhibited to an extent when IGF-1 was prevented from binding to its receptor, which has been theorized to stop the growth of keloids and other scar tissue [6].

Conclusion

There would be great clinical importance to screening for some of the biomarkers discussed in this review. Current
research indicates that high levels/activity of the HLA-DRB1 gene, SMAD transcription factors, TGF-β, and specific alleles within the major histocompatibility complex familial have implications in for formation of keloid scars and give good biomarker targets for screening of patients before they undergo elective facial surgery.

To conclude, there are numerous genes and protein markers that have been positively correlated with keloid scarring that can be used clinically to identify patients prone to keloids in the pre-operative stage before facial surgery. Further research and clinical trials would be useful to find a more detailed connection between the different keloid’s biomarkers and how they act as risk factors in the development of this aberrant form of scarring.

References