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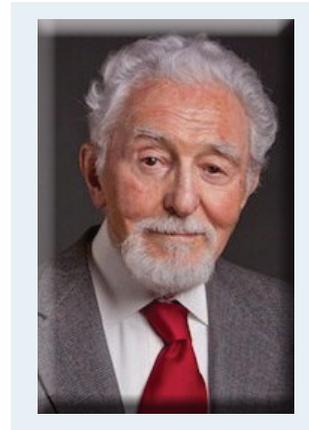
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Analysis of Asian Male Eyebrow by Trichoscopy for a Better Reference on Eyebrow Transplantation

Changing the Cellular Environment Can Improve the Tissue Response of Follicles

News You Should Know: Malpractice Lawsuit Involving Delegation of FUE

In Loving Memory Norman Orentreich, MD December 1922—January 2019



The hair transplant community mourns the death of one of its earliest pioneers, Norman Orentreich.

Dr. Orentreich is widely regarded as the father of modern hair transplantation. In the early 1950s, he was the first to perform the autologous transplantation of hairs from the occipital scalp into balding areas in androgenetic alopecia. These hairs actually grew permanently, a phenomenon he described as donor dominance. He published his results in a landmark article in the Annals of the New York Academy of Sciences in 1959. His discovery was widely echoed in the media and he trained numerous doctors in his New York office, contributing to the popularity and recognition of hair transplantation as a permanent treatment modality for hair loss.

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No Differences in Stress Protein Induction and Proliferative Capability in Follicular Unit Grafts Obtained by Follicular Unit Excision (FUE) and Follicular Unit Transplantation (FUT)

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ABSTRACT

Background: Two techniques are currently used in hair restoration surgery: Follicular Unit Excision (FUE) and Follicular Unit Transplantation (FUT). Both techniques give excellent clinical results but differ in the way the follicular units (FUs) are excised and handled. It has been shown previously that careful excision and handling of FUs are critical for the outcome because of molecular events that occur in FU cells outside of the body.

Materials & Methods: In this study, we compared the two methods with respect to cellular stress response and cellular growth properties by measuring the expression of compartment-specific hair follicle genes, stress protein genes, and proliferation-related genes in cultured cells.

Results & Conclusion: Although molecular changes suggest a cellular stress response during the procedure, we found no significant differences between the FUE and FUT grafts and no alteration of growth capabilities of the hair follicle cells obtained by FUE and FUT.

INTRODUCTION

Currently, two techniques of donor harvesting used for follicular unit (FU) graft isolation are used in hair restoration surgery. Both of the following techniques produce grafts that can be readily transplanted into the recipient area:

1. **Follicular Unit Transplantation (FUT)**, which involves the excision of a strip of occipital skin and

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isolation of FUs in vitro from the strip using a stereomicroscope.

2. **Follicular Unit Excision (FUE)**, which uses special equipment to harvest FU grafts individually and directly from the donor area of the scalp skin.

To elucidate the molecular changes in FU graft cells obtained by either method, we analysed the expression of numerous genes of the cellular stress response. Additionally, the growth properties of FU grafts (measured by monitoring cell cycle-related genes) during culture of the grafts were studied. Furthermore, the expression of compartment-specific genes of the hair follicle was analysed.

A cellular stress response occurs after exposing cells to various external stimuli. Among these, heat, cold, UV light, hypoxemia (oxidative stress), metabolic changes, or toxic metabolites at the cell membrane are well described.¹⁻³ Furthermore, a cellular stress response of cells is also described during changes of tissue homeostasis, for example, during wound healing, tissue remodeling, or mechanical damage.⁴ In human hair follicles, cellular stress response was associated with age-induced hair greying, but the underlying mechanisms have to be further clarified.⁵ The different systems and pathways of intracellular stress proteins are able to influence various important pathways of the eukaryotic cell. They are involved in regulating signal transduction pathways (i.e., regulation of G protein signaling), protein ubiquitination and degradation, messenger ribonucleic acid (mRNA) processing, and other key regulatory systems of the cell. In general, stress-related proteins stabilize cellular reactions under conditions harmful to the cell.

Among the various protein families involved in the cellular stress response, we have focused on proteins shown previously to play a central role in different stress response pathways. In this study, we analyzed the expression of members of three important stress protein families:

1. **The heat shock protein (HSP) family.** This protein system comprises 5 conserved protein families of different molecular weight and involves over 50 protein molecules. The best-known functions of HSPs are their role as molecular chaperons and regulators of protein phosphorylation. Furthermore, they control apoptotic pathways of the cell and therefore play a key role in promoting survival of the cell.¹
2. **Ribosomal proteins (RP).** This protein system is part of the cellular translational machinery responsible for protein synthesis from mRNA. It comprises of 4 ribosomal species with 79 ribosomal proteins. Just like HSPs, these proteins are involved in regulating various physiologic and pathologic processes. Among these, controlling the ribosome assembly and protein translation as well as activating the tumor suppressor p53 pathway in response to ribosomal stress are central.
3. **Regulators of G protein signaling (RGS).** This family comprises about 20 singular proteins, which interact with G protein coupled signal transducing proteins to “fine-tune” the cellular response after activation of G protein coupled receptors. RGS proteins are multifunctional, GTPase-accelerating proteins, which promote

GTP hydrolysis and switch off the G protein signaling pathway. Different sorts of cellular stress (e.g., febrile temperatures, proteotoxic, biochemical or ischemia induced stress) alter transcription of different RGS proteins (RGS2, RGS4, RGS5, and others). It has been reported that the relative amount and ratio of RGS proteins is crucially regulated during the cellular stress response.⁶

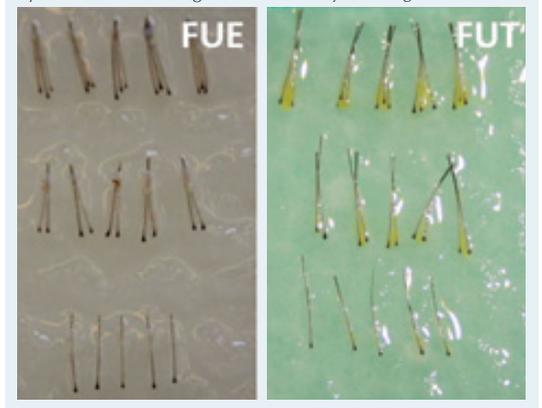
Because the preparation of the FU grafts involves different mechanisms of disturbing tissue homeostasis, the alteration of the expression of cellular stress protein seems likely. Therefore, we analyzed the key proteins HSP90, RPs, and the two RGS proteins RGS2 and RGS4, in FU grafts obtained by FUT or FUE.

METHODS AND RESULTS

Preparation of follicular unit grafts

FU grafts were prepared by standard surgical techniques used at Moser Medical Group for preparation of FU grafts by FUE or FUT. The appearance of the FU grafts which were used for molecular characterization is shown in Figure 1.

FIGURE 1. Macroscopic appearance of FU. FU grafts obtained by FUE (left) and FU grafts obtained by FUT (right).



Compartment-specific hair follicle gene expression

To evaluate the alteration of different compartments of the hair follicle (dermal papilla, epithelial compartments), we measured the expression of compartment-specific genes: alkaline phosphatase (ALP) and versican were used as dermal papilla marker genes and the keratins KRT15 (outer root sheath), KRT25 (inner root sheath), and KRT85 (cuticula) were used as markers for the epithelial compartment. As shown in Figure 2, we found a time-dependent decrease of ALP and versican as reported previously by our group.⁷ No statistically significant differences in compartment-specific mRNA expression between FU grafts from FUT and FUE were found.

Stress protein gene expression

The mRNA of the investigated stress-related proteins showed a distinct pattern. The amounts of HSP90, RPS, and RGS2 mRNA remained essentially unchanged up to 180 minutes after micrograft isolation in both grafts from FUT and FUE (Figure 2). We found a time-dependent decrease of RGS4 in grafts, which is slightly more pronounced in grafts isolated by the FUT method as compared to FUE (fold change to baseline after 30 minutes: FUT 28.44 ± 1.56 vs. FUE 23 ± 2.82 ; Figure 3). This decrease changes the RGS2/

FIGURE 2. Changes of dermal papilla cell and epithelial cell marker mRNA in FU grafts obtained by FUE and FUT. Alkaline phosphatase (ALP) and versican were used as dermal papilla marker genes, the keratins KRT15 (outer root sheath), KRT25 (inner root sheath), and KRT85 (cuticulla). mRNA expression was monitored in fresh FU grafts and in FU grafts stored at room temperature for 30, 90, and 180 minutes.

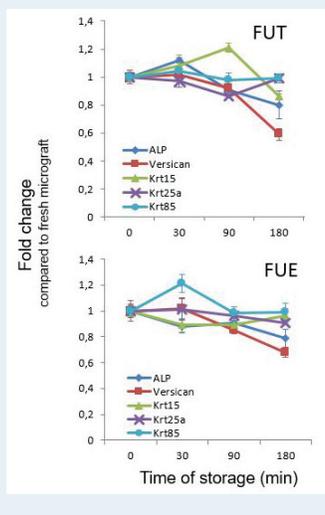
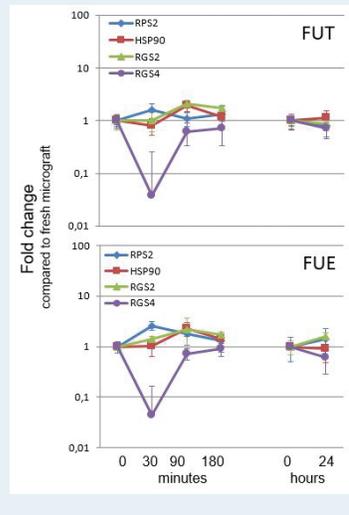


FIGURE 3. Changes of cellular stress protein mRNA in FU grafts obtained by FUE and FUT. Heat shock protein 90 (HSP90), ribosomal protein S (RPS), regulator of G protein signaling (RGS) 2 and 4 mRNA expression were monitored in fresh FU grafts and FU grafts stored at room temperature for 30, 90, and 180 minutes.

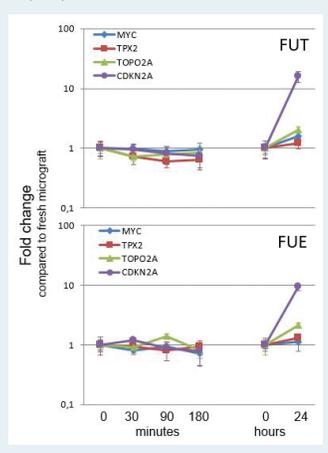


RGS4 relation known to occur during cellular stress and therefore argues for cellular stress in hair follicles in both types of isolation.⁶

Induction of proliferation-related genes during culture

To evaluate the viability and proliferative capability of graft cells after isolation by FUT or FUE, their proliferation-related gene expression was monitored after 24 hours of micrograft culture (Figure 4). Evaluation of the capacity of culture-induced cell proliferation of isolated grafts revealed induction of cyclin-dependent kinase inhibitor 2A (CDKN2A) mRNA in both grafts of FUT and FUE, with a slightly stronger proliferative response of grafts isolated by FUT (fold change to baseline after 24 hours culture: FUT 15.85 ± 0.96 vs. FUE 9.34 ± 1.23; Figure 4).

FIGURE 4. Changes of proliferation-related protein mRNA in FU grafts obtained by FUE and FUT. MYC, targeting protein for Xklp2 (TPX2), DNA topoisomerase 2-alpha (TOPO2A), and cyclin-dependent kinase inhibitor 2A (CDKN2A) mRNA expression was monitored in fresh FU grafts and FU grafts stored at room temperature for 30, 90, and 180 minutes.



We found no significant changes of the constitutively expressed genes MYC, targeting protein for Xklp2 (TPX2), and DNA topoisomerase 2-alpha (TOP2A) in grafts obtained by FUT and FUE.

CONCLUSION

In conclusion, our data demonstrate that during preparation and storage of FU grafts, a cellular stress response occurs. This stress response showed alterations in mRNA expression

of the RGS system, which has been previously demonstrated to occur after mechanical and/or proteotoxic or ischemia-induced cellular stress. However, the stress response in FU graft cells seems to be limited and a good proliferative response of the micrograft cells in tissue culture was observed. The surgical technique used for isolation of the grafts does not influence the type or extent of the stress response. Grafts obtained by FUT and FUE showed the same pattern of mRNA expression of the investigated genes. Furthermore, viability and proliferative response are comparable in grafts obtained by FUT and FUE as demonstrated by mRNA pattern of compartment-related genes and proliferation-related genes.

References

1. Fulda, S., et al. Cellular stress responses: cell survival and cell death. *Int J Cell Biol.* 2009; 2010:1-24.
2. Kültz, D. Molecular and evolutionary basis of the cellular stress response. *Annu Rev Physiol.* 2005; 67:225-257.
3. Galluzzi, L., T. Yamazaki, and G. Kroemer. Linking cellular stress responses to systemic homeostasis. *Nature Rev Mol Cell Biol.* 2018; 19:731-745.
4. Barbee, K.A. Mechanical cell injury. *Ann NY Acad Sci.* 2005; 1066:67-84.
5. Seiberg, M. Age-induced hair greying—the multiple effects of oxidative stress. *Int J Cosmetic Sci.* 2013; 35:532-538.
6. Song, L., and R.S. Jope. Cellular stress increases RGS2 mRNA and decreases RGS4 mRNA levels in SH-SY5Y cells. *Neurosci Lett.* 2006; 402:205-209.
7. Krugluger, W., et al. Evaluation of gene expression patterns in micrografts demonstrate induction of catagen-like processes during storage. *Dermatol Surg.* 2017; 43:275-280. ■

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