

The Relation between ALS and the Skin: A Novel Human *In Vitro* Model to Identify New Biomarkers

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Editorial

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting the motor neurons of the brain and spinal cord causing progressive paralysis and death [1]. ALS patients develop progressive muscle weakness, atrophy, then paralysis and death within 3 to 5 years after the onset of the disease [1,2]. Close to 90% of all ALS cases are sporadic (SALS) while the other 10% represent familial ALS (FALS) [3]. ALS diagnosis is actually based on clinical assessment of related symptoms [4]. As a chronic progressive disease, ALS has a pre-symptomatic period during which the pathological process begins, but motor signs required for the clinical diagnosis are absent. This particularity makes the development of disease-modifying therapies for ALS very difficult as the range, being from 8.0 to 15.6 months between the first symptoms and the diagnosis, is quite long [4]. At this late stage in the development of the disease, numerous motor neurons have already died or gone through degeneration process. The identification of biomarkers in ALS has been a very active area of investigation, employing transcriptional studies, protein profiling in blood and CSF, imaging, and electrophysiological techniques. While these techniques have identified some potential ALS biomarkers, so far none have proven to be clinically useful. Therefore, the discovery of specific biomarkers for ALS is of great importance to help facilitate early diagnosis of the disease as well as monitoring its progression and assess response to existing and future treatments. Both skin and neural tissue come from the same germ layer known as the ectoderm. ALS, as well as other neurodegenerative diseases, are often accompanied by skin changes that appear before the appearance of neurological symptoms [5]. The non-cell autonomous toxicity in ALS has been

well established as there is increasing evidence demonstrating that the convergence of damage developed within multiple cell types, including non-neuronal cells, is crucial to neuronal dysfunction [6-12]. The involvement of other cell types reveals new perspectives to better understand this disease.

Our research team has developed an innovative human-based *in vitro* model to help identifying ALS biomarkers [13]. Using this ALS tissue-engineered skin model (ALS-TES), derived from ALS patient's skin cells, we have shown that it is possible to detect a number of abnormal features uniquely seen in the ALS-TES. As a matter of fact, the ALS-TES model, derived from both FALS-*C9orf72* and SALS patients, presented evident structural abnormalities as an undifferentiated epidermis, cohesive failure of the stratum corneum, abnormal dermo-epidermal junction, delamination, as well as collagen misorganization. In contrast, control-derived TES showed a well-developed and differentiated epidermis and highly organized dermis. Quite interestingly, these abnormal skin defects were detected in pre-symptomatic *C9orf72*-linked FALS patients carrying the G4C2 DNA expansion repeat. TDP-43 cytoplasmic inclusions, a well known pathological signature of ALS, were uniquely detected in the ALS-derived skin [14,15]. In contrast, no TDP-43 abnormalities were observed in the control-derived TES. It is the first time that cytoplasmic TDP-43 inclusions are detected outside

of the nervous system and in non-neuronal cells in any model so far. Consequently, this ALS-TES model could represent a renewable source of human tissue, quickly and easily accessible to better understand the physiopathological mechanisms underlying ALS, to identify predictive biomarkers and help develop innovative tools for disease monitoring and drug screening.

There is a growing body of literature on some apparently unique skin changes that occur in ALS patients [10,16]. The original observation dates back to the nineteenth century when Charcot first observed that there was an absence of bed sore in ALS patients unlike other bedridden patients [17]. There are now several other studies describing physiological skin changes in ALS and other neurodegenerative diseases [5,16]. At the molecular level, skin changes in ALS patients have also been described in case report studies such as cystatin-C, FUS, HGF, IGF-1, IL-6, laminin I, progranulin, TDP-43, TNF- α , ubiquitin, VCP and VEGF immunoreactivity [17-29]. It has also been shown that abnormal level of structural skin proteins such as collagen, elastin and matrix metalloproteinases (MMPs) can be in native skin biopsies taken from ALS patients [30-33]. The extracellular matrix (ECM) stability in the skin of ALS patients, as well as in our ALS-TES model, could therefore be impaired and possibly be used as hallmark in the progression of the disease. In conclusions, skin of ALS patients may presents irregularities and could be of particular interest in the discovery of specific ALS biomarkers.

While these interesting findings reveal that it is possible to detect changes in biopsied skin samples collected from ALS patients, it is difficult at the moment to find any significant correlation between these skin changes and the disease due to the limited size of the biopsies and lack of validations. The usefulness and contribution of IHC in solving problems in pathological anatomy is directly proportionate to the experience of the hands that perform the reactions and also the eyes that interpret the results. Therefore, even though very simple in concept, semiquantitative immunostaining methods requires rigor of execution and may present significant bias [33]. Full validation of IHC results at the biochemical, molecular and protein levels is thus critical in interpretation of results. Our ALS tissue-engineered skin model would therefore provide a unique and innovative model to fully

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study the relation between skin changes and ALS, as well as identifying specific biomarkers for early diagnosis or to follow disease progression. Such validated biomarkers, which can be detected outside the nervous system using easily accessible tissue, have never been described thoroughly in ALS. The identification of such validated biomarkers will not only improve diagnosis and allow to initiate therapy earlier, but also allow stratification in clinical trials and give novel molecular tools to improve clinical trials outcome measures. Conceivably, the application of TES to facilitate the identification of biomarkers for early diagnosis or to monitor disease progression becomes highly attractive and will be of high importance. Skin biopsies of ALS patients could therefore become an important tool for ante-mortem diagnosis of ALS and other neurodegenerative diseases. These will let researchers and clinicians overcome the fact that neurological samples are nearly impossible to get from living patients. The use of skin biopsies taken from ALS patients, and possibly from patients suffering from other neurodegenerative diseases, or ALS-TES as a window into the CNS may therefore represents an original and novative approach with implications that my well extend beyond ALS.

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