Tissue Transglutaminase in astrogliosis: towards improved remyelination

Nathaly Espitia Pinzón and Anne-Marie van Dam

VU University Medical Center, Dept. Anatomy and Neurosciences, Van der Boechorststraat 7, 1081 BT Amsterdam. n.espitia@vumc.nl

Research question and background

Multiple Sclerosis (MS) is a chronic demyelinating disease of the central nervous system in which astroglial cells become hypertrophic, produce various extracellular matrix (ECM) proteins, and interact with ECM proteins to contribute to a non-permissive environment for repair. Tissue Transglutaminase (TG2) expression is enhanced in astrocytes in MS lesions. This enzyme is well-known for its protein cross-linking capacities, and is involved in cell adhesion processes. In this study our aim was to study TG2 expression in MS lesion derived astrocytes versus control astrocytes, and subsequently what the functional consequences of differential TG2 expression were for ECM crosslinking and cell adhesion properties.

Methods and tissues used

To isolate primary astrocytes the provided NBB tissue was chopped and trypsinized for 30 minutes. Tissue was washed and using a percoll grandient astrocytes were isolated from myelin, debris etc. Cells were plated in a T75 cell culture flask. The material we requested from the brain bank was: max. 10 x multiple sclerosis: subcortical white matter with MS lesion (in hibernate medium) and max. 10 x non-demented control: subcortical white matter (in hibernate medium). The tissue we received was (in NBB numbers): 2012-017, 2012-020, 2012-025, 2012-029, 2012-030 and 2012-033.

Results and conclusion

Unfortunately in our hands, the primary human astrocytes showed a very moderate survival/proliferation in culture. Moreover, because of the long culture time necessary to have enough astrocytes, we were confronted with a high incidence of bacterial/yeast contamination in our human primary cultures making them unsuitable to do our proposed experiments with. Other consulted experts confirmed our experiences. All together, we concluded that for our aim, we were not able to culture enough human astrocytes within a short period of time of consistent quality. Therefore, we decided to continue this line of research using human astrocyte cell-lines instead of primary human astrocytes.