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Drexel University College of Medicine

In the tradition of Women's Medical College of Pennsylvania and Hahnemann Medical College

Department of Pathology and Laboratory Medicine

Allison Diamond
Associate Editor, Journal of Visualized Experiments (JoVE)

Dear Ms. Diamond,
Enclosed is a manuscript by Crowe, Bitto, Bhat, Johnson, Trojanowski, Sell and myself entitled: "Identification of cells with markers of cellular senescence in human formalin-fixed, paraffin-embedded brain tissue sections".

Recently we demonstrated for the first time the presence of senescent astrocytes in human brain, during aging and as component of the brain of patients suffering from Alzheimer's disease (Bhat et al. 2012, PLoS One. 2012;7(9):e45069). We have proposed that the presence of senescent astrocytes contributes to the pathogenesis of AD and may represent a link between the aging process and progression of the disease. In previous studies, we identified $p16^{INK4a}$ as a major protein up-regulated during astrocyte senescence (Bitto et al., Exp Cell Res. 2010:2961-8), and we have used this marker to identify senescent astrocytes in the archived postmortem human brain. In the manuscript, we describe the protocol for the detection of $p16^{INK4a}$-positive astrocytes by double immunofluorescence in human formalin-fixed, paraffin-embedded brain sections. The protocol involves several stages including tissue preparation, immunological detection, imaging and quantitation; and we feel that the opportunity to methodologically illustrate and accurately evaluate senescent cells in human brain tissue fits with JoVE's aims and unique multimedia format.

Senescent cells have been shown to accumulate in human tissues during aging and to underlie aging-related disease phenotypes; however, little information is available regarding the detection of senescent cells in human brain tissue. Therefore, we believe that a method to identify and evaluate the proportion of senescent astrocytes in human brain tissues could serve as the basis for investigating the consequences of their presence in a diverse set of pathological brain conditions.

Author contributions: protocol design and standardization EPC, AB, RB, CT. Data analysis: EPC, AB, FBJ, JQT, CS, CT. Manuscript writing: EPC, AB, CT.

I hope you will find our manuscript suitable for publication in JoVE.
Best regards,

Claudio Torres, Ph.D.
Assistant Professor
Mail Stop 435, 245 N. 15th Street, Philadelphia, PA 19102-1192 **TEL** 215-762-7119 **FAX** 215-762-3978