LAMININ PROTEOLYSIS INDUCED BY MMP-9 MODULATES NEURITOGENESIS

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Laminin, a glycoprotein of the extracellular matrix, plays an important role in the axon growing, both in development and in regeneration of the nervous system. In previous work it was shown that the presence and shape of laminin polymers secreted by astrocytes controlled neuritogenesis. On the other way, it is known that glioma cells are highly permissive to neuritogenesis, although they produce metalloproteases which degrade laminin. This study evaluated the role of laminin in the regulation of tumor cell-induced neuritogenesis. Cortical neurons obtained from embryos rat of 14 days (E14) was plated upon: normal astrocytes (NA), glioma cells (C6), polylysin and conditioned medium by NA (CMNA)/ with or without laminin polymers (PLMN) or polylysin and conditioned medium by C6 (CMC6)/ with or without PLMN. The cell morphology was evaluated by immunostaining of β-tubulin and neurite lengths were analyzed using ImageJ Software. The characterization of metalloprotease activity of conditioned medium and laminin degradation were evaluated by zymography and electrophoresis, respectively. The results showed that the neurite outgrowth in neurons co-cultured on C6 is compatible with that shown on NA. However, it was not observed the presence of arrays of laminin on C6 cells. Upon polylysin, the growth was reduced by about 60% as compared to the growth on the cells. Adding of PLMN to the conditioned media regained neurite outgrowth promoted by the cell layer. Specifically, when PLMN was added to the CMC6, the neurite outgrowth was increased by 30% when compared to the effect on CMNA. The zymography associated with electrophoresis characterized the presence of the 110kDa metalloprotease (MMP-9) in CMC6. The proteolytic activity of conditioned medium produced a 30kDa fragment of laminin. The neurite outgrowth was not influenced by the proteolytic activity of CMC6. Furthermore, the data suggest that fragmentation PLMN can increase their neuritogenic activity.

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