Migratory and invasive behaviour of cancer stem cells from glioblastoma patient.

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Due to the highly dismal prognosis of brain tumors, particular attention has been focused on cancer stem cells (CSCs) isolated from glioblastoma multiforme (GBM). GBM CSCs are resistant to radio- and chemotherapy thus making futile conventional therapies. Issues regarding CSC movement are important in neurosphere biology as cell–cell or cell–environment interactions may have significant impacts on CSC differentiation and contribute to the heterogeneity of the neurosphere.

Despite the growing body of literature data on the biology of brain tumor stem cells, floating CSC-derived neurospheres have not been fully characterized from a morphological and ultrastructural point of view. Here we report the first study of the migratory and invasive behaviour of cancer stem cells: a CSC line (L0627) obtained from a patient with diagnosis of primary glioblastoma [1] was employed.

Unlike cells from stabilized tumor lines, after the contact with Matrigel™ CSCs immediately began to cross the membrane and, as observed by scanning electron microscopy (SEM) and laser scanning confocal microscopy (LSCM), after just half an hour of incubation cells were able to pass through 80 μm film (Fig. 1 A). As demonstrated by SEM observations, CSCs did not reorganize in neurospheres but adhere to the film and immediately begin to penetrate it (Fig. 1 B). The apparent absence of proteolytic degradation was strongly suggestive of a type of amoeboid movement, already found in stabilized tumor cell lines showing high invasive potential. On the lower side of the filter it was possible to find cellular elements of different morphology and size, characterized by a surface completely covered by the extracellular matrix. After 1 and 3 h the cells appeared either spread on the film or sunk into it; Matrigel™ showed a lot of cracks not previously observed (Fig. 2 A). On the lower side of the membrane large clusters of cells entirely covered by the matrix were also visible (Fig. 2 B). It was difficult to distinguish between fibers and cells and this suggested that CSCs firstly penetrated the matrix, then they invaded and colonized it, becoming all one with it.

In conclusion, during the process of invasion, CSCs displayed very heterogeneous behaviours and morphology, never observed in previous experiments. The coexistence of many different shapes and behaviours makes difficult the interpretation and discussion of these data. The literature is poor of information, and our laboratory is one of the first to study the motility of CSCs with this kind of methodologies.

1. R. Galli et al., Cancer Res. **64** (2004) p.7011.

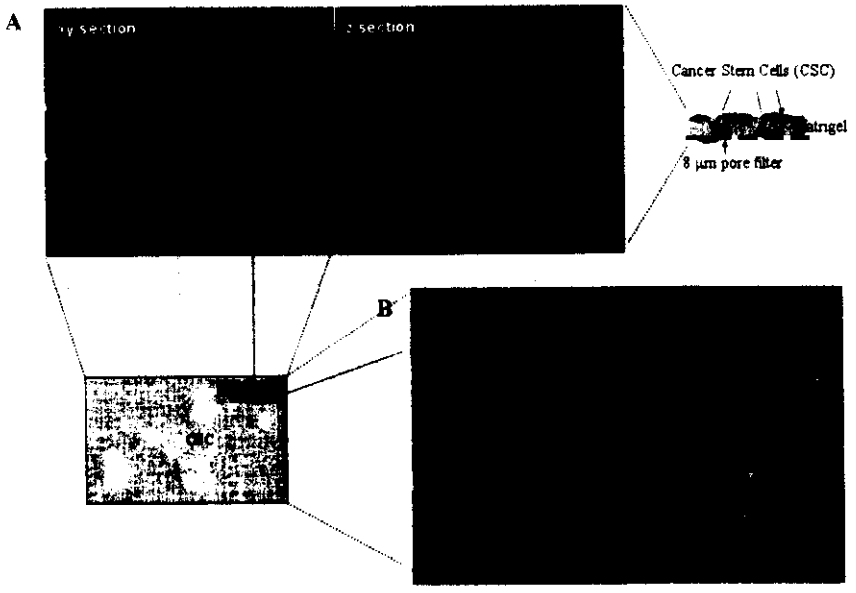

Figure 1. A) LSCM images of CSC invading Matrigel™ thickness 80 μm. B) SEM image shows GBM CSCs that adhere to the film after half an hour and immediately begin to penetrate it.

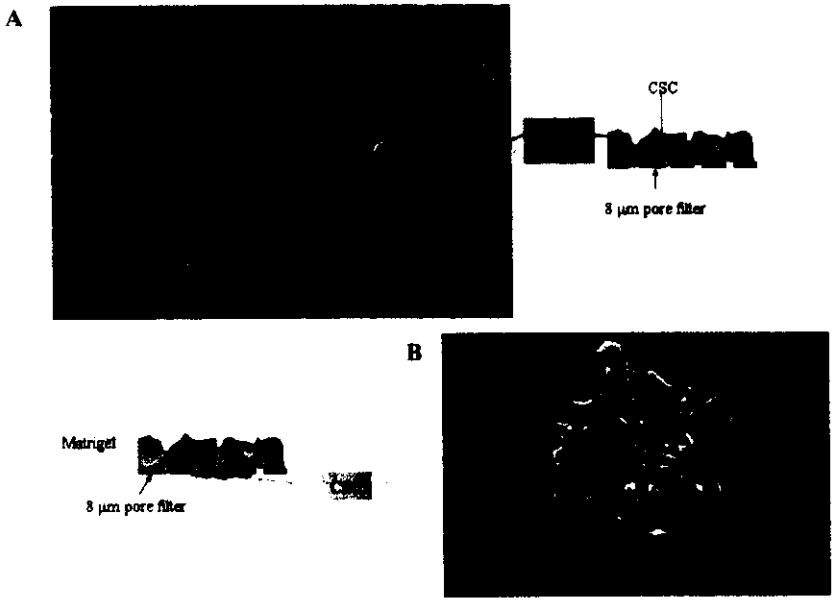

Figure 2. SEM images of GBM CSCs after 3 hours of incubation with Matrigel™ on the upper side (A) and on the lower side of the membrane (B).