

Brain tumor initiating cells show sensitivity towards Notch inhibition



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Background

- Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults
- Brain tumor initiating cells (bTICs) are a pool of neural stem cell (NSC)-like cells found in different grades of glioma
- bTICs might be responsible for tumor-initiation, -progression, treatment resistance and relapse
- Notch signaling is important for maintaining an undifferentiated pool of normal NSC and in determination of cell fate (Figure 1)
- Notch signaling is indicated a functional role in GBM and thereof derived bTICs

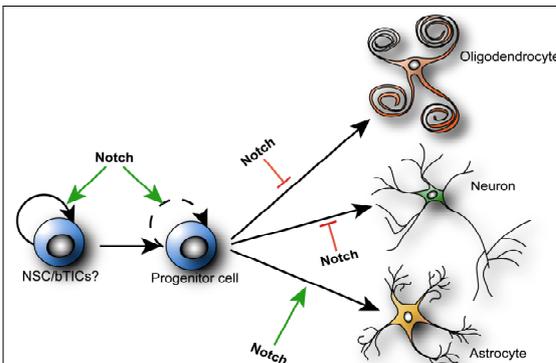


Figure 1: Active Notch signaling is important for the self-renewal of normal NSC and possible of bTICs. In addition, an active Notch cascade is central for the terminal differentiation of astrocytes and on the contrary inhibits the maturation of neurons and oligodendrocytes

Aim

Investigate the significance of Notch expression and activation in GBM stem-like cultures originating from human primary GBM

Material and Methods

- Neurosphere cultures were established from human derived primary GBM xenografts and cultured in NB-media: Neurobasal –A media supplemented with b27, L-glut, EGF, bFGF (Invitrogen) and LIF (Chemicon). G1, G2 and G3 are three different primary GBM tumors and their corresponding xenografts and neurosphere cultures
- Notch inhibition was accomplished using the γ -secretase inhibitor DAPT (Calbiochem) dissolved in DMSO (Sigma). 5 μ M DAPT was used, unless otherwise mentioned. Equal volumes of DMSO was used as a control
- Protein expression was determined by Western blot analysis (WB)
- mRNA expression was analyzed by Quantitative Real-Time Polymerase Chain Reaction (q-RT-PCR)

Results

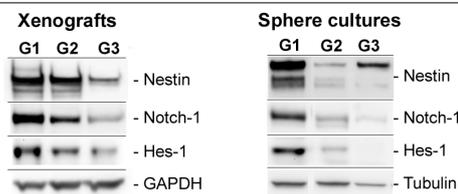


Figure 2: WB detection of the Notch-1 receptor, the Notch target Hes-1 and the NSC marker Nestin in GBM xenograft tissue and Neurosphere cultures.

Results

Effect of Notch inhibition on Hes-1 mRNA expression

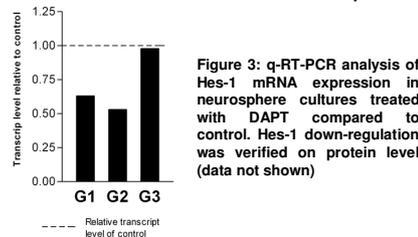


Figure 3: q-RT-PCR analysis of Hes-1 mRNA expression in neurosphere cultures treated with DAPT compared to control. Hes-1 down-regulation was verified on protein level (data not shown)

Notch inhibition reduces primary sphere formation

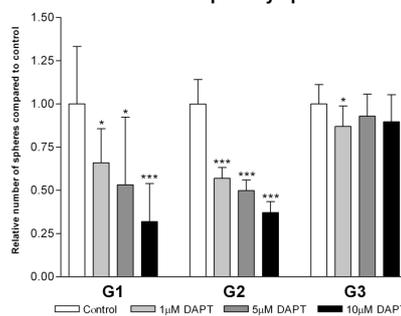


Figure 4: Primary sphere assay. Cells from acutely dissociated xenograft tissue were plated in NB-media with different concentrations of DAPT. Number of spheres was evaluated at day 14

Notch blockage induces differentiation of sphere cells

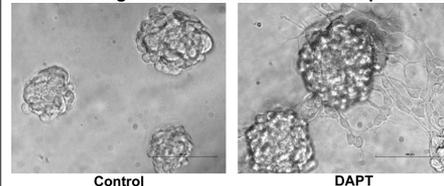


Figure 5: Spheres formed in NB-media during DAPT treatment had a tendency to adhere to the culture plate and cells with a differentiated morphology migrated away from the spheres

Notch inhibition alters the differentiation pattern

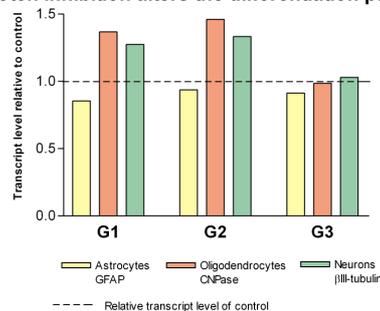


Figure 6: Sphere cells were plated in serum-containing media in order to induce differentiation during concurrent DAPT treatment. Afterwards q-RT-PCR analysis of markers for the three neural lineages was performed

Notch blockage reduces neurosphere cell migration

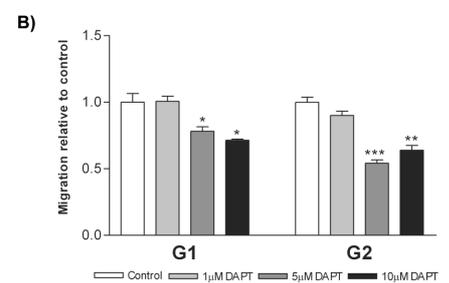
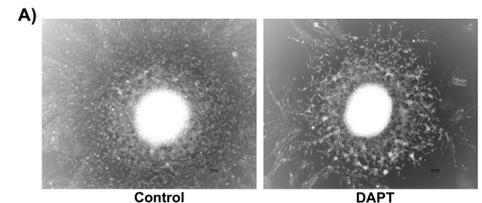


Figure 7: A) When whole spheres were transferred to serum-containing media, they adhered to the culture plate and cells began to migrate away from the sphere. B) Sphere cells were pretreated with different concentrations of DAPT and subsequently seeded in a modified Boyden chamber. The amount of migrated cells was quantified by MTT-staining

- Expression of the Notch-1 receptor correlated with Hes-1 and Nestin expression. G1 expressed the highest level of all three markers, while G3 expressed the lowest level (Figure 2)
- Notch inhibition was verified by down regulation of Hes-1 mRNA (Figure 3)
- The primary sphere-forming potential was significantly reduced upon Notch inhibition (Figure 4)
- Notch inhibition seems to promote differentiation of sphere cells (Figure 5)
- Notch blockage led to altered differentiation pattern, in accordance with the established role of Notch in cell fate decisions (Figure 6 and figure 1)
- Cell viability was hampered when Notch signaling was inhibited (Data not shown)
- The migratory potential of sphere cells was reduced upon Notch inhibition (Figure 7)
- The overall effect of DAPT treatment was more pronounced in G1 and G2 cultures when compared to G3 cultures

Conclusion

Notch signaling contributes to the NSC-like character and the malignant phenotype of bTICs, when these display dysregulated Notch pathway activation. It might be possible to target bTICs in human GBM through the Notch signaling pathway.