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

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). 5µM 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
- Notch blockage reduces neurosphere cell migration
- 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 downregulation of Hes-1 mRNA (Figure 3)
- The primary sphere-forming potential was significantly reduced upon Notch inhibition (Figure 4)
- Notch inhibition appears 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.