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Expression and Regulation of CD133 in Glioblastoma

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Glioblastoma (GBM) represents the most common and malignant primary tumor of the central nervous system. Increasing evidence has been gathered supporting the existence of tumor stem cells in GBM. To date, the most widely reported marker for the isolation of this tumor sub-population in GBM is CD133. However, the natural function of CD133 and how it is regulated still remain unknown. Therefore, the present work aims to further clarify the possible role of CD133 in GBM.

In vitro experiments with GBM stem cell (GSC) lines and conventional GBM cell lines were performed. RT-PCR, immunofluorescence staining, FACS analysis and con-focal microscopy were performed to study if there were any differences in CD133 expression between conventional GBM cell lines and GSC lines. Quantitative PCR, FACS analysis and Western blot were performed to observe the effect of differentiation or hypoxia on the expression of CD133 in GSC lines and to investigate the possible regulatory mechanism for its expression.

It was found that the AC133 epitope could only be detected in GSC lines, whereas conventional GBM cell lines were negative. This distinction was independent of CD133 mRNA or protein expression levels, since CD133 protein was also expressed in conventional GBM cell lines. However, an ER or Golgi sub-localization of the intracellular CD133 protein in the conventional GBM cell lines was detected. No specific splice variants were detected in

AC133-positive GSC lines or in cell lines with different intracellular distribution of the CD133 protein.

Differentiation resulted in a decreased AC133 detection in GSC lines, whereas CD133 protein expression levels remained equal in two of three cell lines. This decreased AC133 detection on the cell surface did not result from a shedding of AC133. The decreased AC133 detection upon differentiation was due to a regulation occurring at the post-translational level. Besides this post-translational regulation, Some GSC lines showed a regulation on the transcriptional level, since coinciding with decreased AC133 detection upon differentiation, down-regulated CD133 mRNA was also observed.

Hypoxia resulted in the increased AC133 detection in GSC lines, whereas CD133 protein expression displayed no apparent change. The up-regulated AC133 epitope detection under hypoxia in GSC lines again resulted from the post-translational regulation.

Based on these results, it can be concluded that CD133 mRNA is widely distributed both in differentiated cells and undifferentiated cells in GBM, and CD133 protein expression is consistent with its mRNA expression, whereas the AC133 epitope is restricted to GSCs. Besides regulation of CD133 occurring at the transcriptional and the translational level, post-translational regulation constitutes the more common regulatory mechanism for AC133 expression under different environmental stimuli, i.e. differentiation or hypoxia.