4. Discussion

4.1 Summary

In this PhD thesis we analyzed the expression pattern of specific glycoepitopes, as a part of the ECM, recognized by the mAbs 487$^{LeX}$, 5750$^{LeX}$, and 473HD as biomarkers during early human development. We provide the evidence that these specific surface antibodies can be utilized to identify distinct stages during neural differentiation of hiPSCs. In this study we focused on six different stages, namely: hiPSCs, hEBs, hNAs, hR-NSCs, hSR-NSCs, and hNSCs$^{FGF-2/EGF}$. The most important findings are summarized in Figure 22.

At the pluripotent stage, 487$^{LeX}$- and 5750$^{LeX}$-epitopes were expressed by hiPSCs. Interestingly, only some cells at the edges of the colony were positive. In comparison, the 473HD antibody labeled more cells at the periphery of the hiPSCs colony. At hEB and hNA stage we observed that all antibodies detected these stages in a different manner. Whereas the expression of 487$^{LeX}$ and 5750$^{LeX}$ was very strong at hEBs stage, it seemed to be down-regulated at hNA stage. In contrast, the DSD-1-epitope, recognized by mAb 473HD, is expressed only in the outer part of hEBs. Also at hR-NSCs stage, all three mAbs behave differently. Both LeX antibodies revealed positive signals, at the marginal zone of the rosettes and in the central lumens of rosettes. The DSD-1-epitope could be weakly identified. In addition, LeX- and DSD-1-epitope were expressed by a subpopulation of “flat” cells around the rosettes. Remarkably, after isolation of rosettes we detected a shift of the glycan expression within hSR-NSCs. We show that both LeX-type glycans and the DSD-1-epitope were expressed at this stage of differentiation. However, the LeX glycan motifs were completely co-expressed with Nestin, whereas the 473HD-epitope was restricted to the middle of the small rosettes. In a further experiment we could show that only a subpopulation of hNSCs$^{FGF-2/EGF}$ expressed the LeX- and DSD-1-epitope. Thereby we made an interesting observation. The LeX-type glycans seemed to be down-regulated, whereas the DSD-1-epitope was highly expressed at this stage of differentiation. However, Western blot analysis revealed a general down-regulation of all epitopes. We also examined the importance of the CS-GAG chains by ChABC-induced degradation. We could show that the subpopulations of cells are not significantly altered after ChABC treatment. However, we demonstrate that the number of PH3-positive M- and G2-phase cells was significantly reduced after digestion of CS-GAG chains.
Figure 22: A schematic summary of the surface epitope expression during human neural induction. (A) The mAbs 487\textsuperscript{LeX}, 5750\textsuperscript{LeX}, and 473HD can be used to detect different stages during neural induction. Each stage of differentiation shows an antibody-specific expression pattern. The graphical abstract represents the results from the immunostainings. (B) The expression of the glycans is differently regulated with ongoing differentiation. Thereby, epitopes of the mAbs 487\textsuperscript{LeX} and 5750\textsuperscript{LeX} behave similarly. Graph illustrates the results from the Western blot analysis and indicates a model for the regulation of the epitopes.
Finally, we identify some potential LeX carrier molecules that are highly enriched in the matrix. RPTPβζ/Phosphacan, Tnc, and LRP1 were identified as possible carrier proteins during neural differentiation.

In light of these results, this study provides new insights into the temporal regulation of glycoconjugates during early human development. Furthermore, we show that mAbs such as 487LeX, 5750LeX, and 473HD serve as promising tools to identify specific stages during human neural differentiation.