Antibodies Against Glycotargets as Novel Platform Approach to Address Unmet Needs in Cancer Therapy


Background

Highly potent therapeutic approaches require clean targets. However, the majority of antibodies in clinical development or approved for cancer therapy address protein targets that are only overexpressed in cancer and yet often show significant expression in healthy organs. Besides protein expression, glycosylation is strongly altered in cancer, reflecting changes in tumor metabolism. Aberrant glycosylation is reasoned by mutated or mislocated glycosyltransferases, glycosidases, substrates and chaperones, giving rise to truncated or highly fucosylated or sialylated glycans. Therefore, tumor-associated carbohydrates like the Thomsen-Friedenreich (TF), Thomsen novelle (Tn) or sialylated Tn (sTn) antigen are expressed on proteins in different carcinomas, leukemias, lymphomas and their metastases.

To increase the tumor-selectivity of protein-targeting antibodies, we developed antibodies against protein/carbohydrate combined epitopes (GlycoTargets), which bind their protein target only in presence of tumor-associated carbohydrates and thereby avoid binding to the protein if it is expressed in healthy tissues.

Methods

The general presence of tumor-associated carbohydrates in cancerous tissues was analyzed using immunohistochemistry. HPLC and mass spectrometry (MS) or bioinformatic prediction was applied to further confirm their presence on specific proteins. Different cell lines were developed and characterized by ELISA and MS to recombinantly express proteins carrying distinct carbohydrates. These GlycoTargets were used for immunization and antibody discovery. Specificity, glycosylation dependency and cell binding properties of antibody lead candidates were characterized by ELISA, flow cytometry and immunohistochemistry on tumor and healthy tissues.

Results

We have identified 220 potential GlycoTargets using bioinformatic predictions or cellular screenings. Using our proprietary cell lines as toolbox for antigen production, we were able to generate highly pure and fully characterized GlycoTargets for tailored immunization approaches and antibody screenings. Two case studies show that our approach is suitable to generate antibodies that bind to the protein of interest only if a specific tumor-associated glycosylation is present and do not cross-react with the non-glycosylated protein or the glycan on irrelevant carrier-proteins. Due to this glycan dependency, our antibodies show markedly decreased off-tumor binding. We will show that our antibodies lack unwanted binding to healthy immune cells in contrast to conventional anti-protein antibodies and stain different cancer indications but not related normal tissues.

Conclusions

Our approach is suitable to target protein/carbohydrate combined epitopes with specific antibodies. With proper tools for target identification, antigen production, immunization and screening, a platform was installed to generate antibodies with markedly decreased off-tumor binding. The increased tumor selectivity may improve safety for highly potent therapeutic approaches like ADCs, CARs or radiopharmaceutics.

REFERENCES
