Characterization of CD44v6 as a potential target for newly engineered antibody-based immunotherapy for bladder cancer.

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Abstract

The mayor challenge of antibody efficacy as targeted cancer therapy tool consists in the selection of specific targets on tumor cells as well as the appropriated stimulatory signals to achieve correct cellular activation in the correct place at the correct time. Overexpression of CD44v6, a splice variant of CD44 glycoprotein, has been found in several tumors and has been associated with tumor progression. CD44v6 expression has also been proposed to be restricted to tumors, which emphasizes its potential as diagnostic biomarker and therapeutic target.

The objective of this project is to offer patients more individualized treatments and improve immunotherapy success rates by developing newly engineered antibody-based molecules able to direct immune cell infiltration and tumor cell specific targeting by effector cells.

CD44v6 expression was analyzed in different stage & grade bladder cancer samples using previously constructed TMAs. The results indicate an association between tumor stage & grade and CD44v6 expression, with increased expression found in higher stage & grade tumors. We also observed an interesting variability in CD44s and CD44v6 receptor expression among multiple human BC cell lines, some of them showing significantly higher CD44s than CD44v6 expression and viceversa. We selected one cell line with CD44s>CD44v6 and one cell line with CD44v6>CD44s expression from which we isolated CD44low, CD44high and CD44v6high cell populations. Differential expression of CSC-related genes in these different cell populations has been suggested. RNA sequencing is being performed on J82 and RT112 parental cells as well as CD44low, CD44high and CD44v6high cell populations, representing the 1-5% cells with the lowest CD44s, highest CD44s and highest CD44v6 expression respectively. Additionally, we successfully produced and purified three variants of recombinant single-chain variable fragment (scFv) antibody recognizing CD44v6, of which one is selected for extensive evaluation by in vitro and in vivo experiments. Large-scale production of the selected scFv CD44v6 variant resulted in a yield of 2.60mg and 25.25mg per liter with purity >90% for the scFv CD44v6 antibody in native and denatured condition, respectively. We also started the construction of chimeric antigen receptor (CAR)-NK cells expressing CD44v6. Besides, we confirmed CD44v6 expression in primary tumors derived from our mouse models.

Conclusively, we explore CD44v6 as potential target for newly engineered antibody-based immunotherapy and expect to define their potential in the treatment of specific BC subtypes. We also started the construction of CAR-NK cells, expressing the anti-BC tumor antibody fragment as moiety for tumor targeting.

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