Role of the complement system in cancer development and anticancer therapy.

Converting pathogenic gain of function variants of complement factor B into enhancers of anti-CD20 therapy.

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Abstract

Anti-CD20 monoclonal antibodies (mAbs) rituximab and ofatumumab, approved for treatment of B cell malignancies, are potent activators of the classical complement pathway. Since complement exhaustion and overexpression of complement inhibitors by cancer cells diminish their therapeutic potential, targeting of membrane complement inhibitors by function-blocking antibodies and supplementation with fresh frozen plasma were proposed as strategies to overcome tumor cell resistance.

We propose a novel approach, which utilizes hyperactive variants of complement convertase forming proteins. Employing HEK293 Freestyle eukaryotic expression system we produced several mutants of factor B, which were previously described in a literature as the gain-of-function variants. In order to simplify the purification process all proteins were 6xHis-tagged at C-terminus and such modification did not influence their specific activity as proven by comparison with purified, native protein isolated from serum.

The proof of our concept is exemplified by factor B (FB), a component of alternative C3/C5 convertases which augment mAbs activated reaction by a positive feedback mechanism called amplification loop. In contrast to further increase of ofatumumab concentration, addition of quadruple gain of function FB mutant p.D279G p.F286L, p.K323E p.Y363A results in significantly increased complement mediated lysis of tumor cells normally resistant to ofatumumab and complete lysis of moderately sensitive cells.