Title: Characterization of Preclinical Models for Oncolytic Adenoviruses

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Oncolytic adenoviruses (OAds) represent a promising avenue in cancer therapy, selectively lysing cancer cells and sparing healthy tissue. They enhance antitumor immunity by inducing immunogenic cell death and disrupting immunosuppressive environment, with intravenous delivery targeting metastases for systemic treatment. However, the clinical advancement of OAds is hindered by the lack of reliable animal models that accurately predict the therapeutic and immunomodulatory effects of OAds, especially upon systemic delivery. Effective preclinical models should support OAd replication, mimic immune responses triggered by OAds, and accurately represent the interaction of OAds with blood components. Standard murine models, however, don't support replication of human adenoviruses, resulting in nonfunctional progenies. Additionally, the absence of CD46 and human desmoglein-2 (hDSG2) receptors in mice and hamsters prevents the binding of species B adenoviruses, like Ad3 and Ad11, affecting chimeric OAd vectors such as Ad5/3. This research aims to identify suitable preclinical models for OAd evaluation. Recent findings indicate that porcine cells and tissues support Ad5 and Ad5/3 vectors' binding, transduction, and replication, comparably to human tissues. This study examines the efficacy of species B, C, and D OAds across various mammals, including mice, hamsters, pigs, dogs, and non-human primates, particularly focusing on systemic delivery and interactions with blood factor X (FX), crucial for adenoviral transduction. Previous studies show human FX enhances cell transduction in species C Ads. Our results reveal porcine and canine FXs significantly boost Ad5, Ad5/3, and Ad6 transduction in CAR-, hDSG2-, and CD46-deficient CHO cells, aligning with human FX effects. Conversely, Ad26 and Ad3 transduction is unaffected by any FX variant, while Ad11 transduction efficiency improves with human and porcine FX. Ongoing research includes Ad types' hemagglutination analysis with mammalian RBCs. We found porcine, murine, and hamster RBCs do not hemagglutinate with the studied Ad types, unlike human RBCs, which react with Ad5, Ad6, AdRGD, and Ad26. Interestingly, only Ad11 causes hemagglutination with non-human primate RBCs (cynomolgus and rhesus macaque). This study aims to deepen the understanding of OAds' therapeutic, immunomodulatory, and safety profiles to aid their clinical translation.