Title: Antitumor effect of CDX2 promoter-controlled oncolytic adenovirus in esophageal adenocarcinoma

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Background: Esophageal adenocarcinoma (EAC) is the most common subtype of esophageal cancer in the US and its incidence has risen dramatically in the last few decades. EAC mostly occurs from Barrett's esophagus (BE) and bile acids reflux into distal esophagus plays an important role in the progression from BE to EAC. CDX2 is reported to be implicated in development of BE and bile acids exposure increases CDX2 expression in BE as well as EAC, although normal squamous esophagus doesn't show CDX2 expression. In this study, we generated CDX2 promoter-controlled oncolytic adenoviruses (OAds). We evaluated antitumor effect of CDX2 promoter-controlled OAd with adenovirus 5/3 chimeric fiber (Ad5/3-CDX2) for EAC.
Methods: Expression of CDX2 in cell lines (OE19 (EAC), Caco2, RH30, and GIST-T1) was analyzed by RT-PCR. We obtained CDX2 overexpress cells by transfecting pCMV-GLI1 plasmid to OE19 (OE19+GLI1). We made replication deficient Ad5/3-CDX2-GL3B (luciferase reporter gene) to assess the CDX2 promoter activity in cell lines by luciferase assay. We then constructed replication competent Ad5/3-CDX2 and evaluated killing effect in cell lines. Antitumor effect of Ad5/3-CDX2 was assessed in mouse model with OE19 subcutaneous tumor treated with intratumoral injection of viruses. Using subcutaneous tumor samples, the viral replication was analyzed.
Results: OE19 and Caco2 showed high expression of CDX2, but not RH30 and GIST-T1. We also confirmed that deoxycholic acid (DCA) exposure induced higher expression of CDX2 and GLI1 in OE19, and OE19+GLI1 cells had overexpression of CDX2. In OE19 and Caco2, Ad5/3-CDX2-GL3B showed increased CDX2 promoter activity, whereas the promoter was not activated in RH30 and GIST-T1. The promoter activity of Ad5/3-CDX2-GL3B was enhanced in both OE19 exposed by DCA and OE19+GLI1 cells. In crystal violet and MTS assay using replication competent viruses, Ad5/3-CDX2 showed killing effect in OE19 and OE19+GLI1 but not RH30. The killing effect was higher in OE19+GLI1 than OE19. In vivo experiment, Ad5/3-CDX2 significantly suppressed tumor growth and the antitumor effect was similar to that of Ad5/3 (with normal promoter: positive control). Ad5/3-CDX2 replicated enough in OE19 subcutaneous tumor after the treatment.
Conclusion: We newly generated CDX2 promoter-controlled OAd that showed antitumor effect for EAC. Our findings demonstrated that specific antitumor effect of Ad5/3-CDX2 has potential for novel therapeutic option targeting EAC.