Title: CDX2 promoter-controlled OAd suppresses tumor growth and liver metastasis of colorectal cancer

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Colorectal cancer (CRC) is the second leading cause of cancer death worldwide and liver metastasis (CRLM) is the most common among its distant metastases. We have recently generated a CDX2 promoter-controlled oncolytic adenovirus (Ad5/3-pCDX2) that showed an anticancer effect for CDX2-positive upper gastrointestinal tumors. Here, we reported the anticancer effect of Ad5/3-pCDX2 for CDX2-positive CRC and CRLM, and its combination efficacy with 5-fluorouracil (5FU) in vitro and in vivo. We used HT29 as CDX2-positive, and LS174T and SW480 as CDX2-negative CRC cell lines. Without 5FU, Ad5/3-pCDX2 killed

HT29 but not LS174T and SW480 cells. In vitro 5FU exposure upregulated CDX2 mRNA levels in all three cell lines. 5FU combination enhanced the cytocidal effect and virus replication of Ad5/3-pCDX2 in CDX2-negative LS174T. In mouse xenograft models, Ad5/3-pCDX2 monotherapy suppressed the HT29 subcutaneous tumor growth compared to the control group. 5FU plus Ad5/3-pCDX2 combination therapy showed a remarkable antitumor effect over the efficacy of Ad5/3-pCDX2 monotherapy. In the LS174T subcutaneous tumor, although Ad5/3-pCDX2 monotherapy did not show an antitumor effect, 5FU plus Ad5/3-pCDX2 combination therapy significantly suppressed the tumor growth, compared to the Ad5/3-pCDX2 monotherapy. In mice with HT29 liver metastasis, intrasplenic injection of Ad5/3-pCDX2 induced the virus replication in liver tumors and thus successfully attenuated tumor growth. In conclusion, Ad5/3-pCDX2 showed a significant anticancer effect that was enhanced by 5FU treatment in not only CDX2-positive but also negative CRCs. Ad5/3-pCDX2 is a promising therapeutic modality for metastatic CRC such as CRLM.