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Expression of the reverse transcriptase domain of telomerase reverse transcriptase induces lytic cellular response in DNA-immunized mice and limits tumorigenic and metastatic potential of murine adenocarcinoma 4T1 cells

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Telomerase reverse transcriptase (TERT) is a classic tumor-associated antigen overexpressed in majority of tumors. Several TERT-based cancer vaccines employing peptides, DNA and CAR technology are currently in preclinical or clinical trials. The most successful offer partial immune control of tumor growth, but the immune correlates of anti-tumor activity remain largely unknown. We aimed to develop DNA vaccine based on TERT of rat, to overcome immune tolerance, and characterize fine specificity and lytic potential of anti-TERT immune response in a mouse model. TERT gene optimized for expression in mammalian cells was designed, synthesized and cloned into DNA vaccine vector as such (TERT DNA) or with hemagglutinin tag (TERT-HA DNA).

Groups of BALB/c mice (n=5) received TERT DNA, or TERT-HA DNA or empty vector in prime, and same DNA mixed with plasmid encoding firefly luciferase (Luc DNA) in boost. DNA was delivered by intradermal injections followed by electroporations. Photon emission from booster sites was assessed by *in vivo* bioluminescent imaging. Two weeks post boost, mice were sacrificed, their CD4+ and CD8+ T cells were assessed by flow cytometry for production of IFN-γ, IL-2 and TNF-α in response to stimulation with TERT-derived peptides. Mouse sera were screened for antibodies against the peptides and recombinant reverse transcriptase (RT) domain of TERT (rtTERT). In 12 days after the boost, photon emission from injection sites in TERT/TERT-HA DNA immunized mice declined 100-fold compared to that in the vector-immunized mice, indicating elimination of TERT/Luc-expressing cells. By experimental end-point, all TERT/TERT-HA DNA-immunized mice developed strong cellular and antibody response against epitopes located at the N-terminus of TERT and rtTERT domain. IFN-γ/IL-2, IFN-γ/TNF-α and IFN-γ/IL-2/TNF-α production by CD8+ and CD4+ T cells specific to rtTERT peptides correlated with the loss of bioluminescence from booster sites pointing at the immune clearance of TERT/Luc-coexpressing cells. DNA encoding rtTERT was recloned into lentiviral vector, further used to transduce murine adenocarcinoma 4T1luc2 cells. Expression of rtTERT by 4T1luc2 tumors reduced tumor growth and metastatic activity of 4T1luc2 cells. Murine mammary gland adenocarcinoma 4T1luc2 cells are highly aggressive, forming large tumors and numerous metastases in multiple organs of syngenic BALB/c mice. Growing tumors induce strong CD4+ and CD8+ T cell response against autoepitopes of telomerase reverse transcriptase (TERT), positively correlating with tumor growth. Expression by murine adenocarcinoma cells of the reverse transcriptase domain of telomerase reverse transcriptase (rtTERT) of rat significantly reduced their capacity to form tumors and generate metastasis, while not affecting their in vitro growth. Mice with restricted growth of rtTERT-expressing tumor cells mounted immune response against CTL epitopes of rtTERT and suppressed autoimmune T-helper and CTL response to autoepitopes of murine TERT. This advances RT domain as a key component of therapeutic cancer vaccines based on TERT. Acknowledgements LZP 2018/2-0308, and RFBR grants 17-54-30002 and 20_04_01034.