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INTRADERMAL DNA IMMUNIZATION OF MICE WITH RAT TELOMERASE REVERSE TRANSCRIPTASE FOLLOWED BY ELECTROPORATION INDUCES MULTIFUNCTIONAL IMMUNE RESPONSE AGAINST ITS REVERSE TRANSCRIPTASE DOMAIN

Jansons Juris[1,2], Kurlanda Alica[1], Skrastina Dace[2], Petkov Stefan[3], Latyshev Oleg[4], Starodubova Elizaveta[5], Ivanova Olga[5], Hippe Laura[1], Kholodnyuk Irina[1], Ivanov Alexander[5], Gordeychuk Ilya[4,6,7], Isaguliants Mara[1,3,4,6]

1. Rīga Stradiņš University, Riga, Lativa;
2. Latvian Biomedical Research and Study Centre, Riga, Latvia;
3. Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden
4. N.F. Gamaleya National Research Center for Epidemiology and Microbiology, Moscow, Russia;
5. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia;
6. Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences, Moscow, Russia;
7. Sechenov First Moscow State Medical University, Moscow, Russia.

Background Telomerase reverse transcriptase (TERT) is a universal tumor associated antigen (TAA). Its overexpression in cells supports continuous their division and promotes malignant transformation. TERT, a highly conserved protein, presents an attractive target for therapeutic cancer vaccines. It forms the basis of a number of therapeutic cancer vaccines based on synthetic peptides, naked DNA, CAR technology, currently in preclinical and clinical trials. Despite these efforts, fine specificity of anti-TERT immune response and immune correlates of anti-tumor activity of TERT-based vaccine candidates remain obscure.

Aims To develop new candidate DNA therapeutic cancer vaccine based on TERT, and characterize its immunogenicity and specificity of cellular and antibody response against TERT in a mouse model.

Experimental Synthetic rat TERT (rTERT) DNA was cloned into expression vector pVax and used to immunize BALB/c mice. Mice in groups of 5, received 20 mcg of DNA encoding rTERT, or hemagglutinin-tagged rTERT, or pVax1, and same plasmids mixed 1:1 w/w with DNA encoding firefly luciferase (Luc DNA). Injections were done intradermally on days 1 (prime), 21 (boost), both followed by electroporation (CUY21 EditII, BEX). Bioluminescence from injection sites after the boost was followed by daily in vivo bioluminescence imaging (Spectrum, Perkin Elmer). TERT-specific cellular response at the experimental end-point was evaluated by

multiparametric flow cytometry assessing percent of IFN-γ, IL-2 and TNF-α production CD4+ and CD8+ T cells, and by antibody ELISA using synthetic peptides (SynPeptide Ltd) predicted by IEDB server services to contain promiscuous T- and B-cell epitopes of rTERT.

Results We chose DNA vaccine technology, and designed expression-optimized DNA immunogen based on rat TERT, deviant in amino acid sequence from its mouse and human analogies, to overcome the tolerance. Photon flux from rTERT/rTERT-HA DNA booster sites reduced 100-times week after 2^{nd} injection compared to flux from vector injection sites, pointing at boosting effect of secondary immunization. All TERT/TERT-HA DNA immunized mice developed prominent CD8+ and moderate CD4+ T-cell response against multiple epitopes in reverse transcriptase (RT) domain of TERT (RT-TERT) and two at the N-terminus of TERT. Dual (IFN-γ/IL-2 or IFN-γ/TNF-a) and triple IFN-γ/IL-2/TNF-α production by CD8+ and CD4+ T-cells in response to RT-TERT-derived peptides correlated with the loss of bioluminescence from the injection sites, supporting the concept of rapid immune clearance of rTERT/Luc-coexpressing cells after the boost. Mice developed antibodies against RT-TERT in titer 10^3-10^4 with B-cell epitopes localized at aa 888-916 and 901-928. HA-tag had no effect on anti-TERT immune response.

Conclusions DNA-immunization of mice with rat TERT induces multifunctional lytic CD8+ and CD4+ T cell and also antibody response against its reverse transcriptase domain. Cellular response clears TERT-expressing cells from the sites of immunization. RT domain, not the full-length TERT, may be sufficient for the TERT-based therapeutic cancer vaccines.

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