Therapeutic efficacy of a DNA vaccine targeting the endothelial tip cell antigen delta-like ligand 4 in mammary carcinoma

ABSTRACT
The Notch ligand delta-like ligand 4 (DLL4) is an essential component expressed by endothelial tip cells during angiogenic sprouting. This paper describes a conceptually novel therapeutic strategy for targeting tumor angiogenesis and endothelial tip cells based on DNA vaccination against DLL4. Immunization with DLL4-encoding plasmid DNA by in vivo electroporation severely retarded the growth of orthotopically implanted mammary carcinomas in mice by induction of a nonproductive angiogenic response. Mechanistically, vaccination brought about a break in tolerance against the self-antigen, DLL4, as evidenced by the production of inhibitory and inherently therapeutic antibodies against mouse DLL4. Importantly, no evidence for a delayed wound healing response, or for toxicity associated with pharmacological blockade of DLL4 signaling, was noted in mice immunized with the DLL4 vaccine. We have thus developed a well-tolerated DNA vaccination strategy targeting the endothelial tip cells and the antigen DLL4 with proven therapeutic efficacy in mouse models of mammary carcinoma; a disease that has been reported to dramatically induce the expression of DLL4. Conceivably, induction of immunity toward principal mediators of pathological angiogenesis could provide protection against recurrent malignant disease in the adjuvant setting.
METHODS

Female BALB/c mice, 6 to 8 weeks old, were immunized three times at the indicated intervals, either by intramuscular injection of 80 µg DNA in musculus tibialis or by intradermal injection of 2 x 40 µg DNA immediately followed by electroporation. Intradermal electroporation protocol was performed using the Agile Pulse In Vivo system, as follows:

**Group 1:**
- 2 pulses
- 1125 V/cm
- 50 µs

**Group 2:**
- 8 pulses
- 275 V/cm
- 10 ms

Fig. 3 Targeting DLL4 by vaccination results in excessive formation of nonfunctional blood vessels. (a) Visual inspection of excised D2F2/E2 tumors revealed overt thrombosis in tumors from DLL4-vaccinated mice. (b) Blood vessel density of D2F2/E2 tumors visualized by immunostaining for the endothelial cell marker CD31 (red; n=4/5 for each group). (c) Perfused blood vessels of D2F2/E2 tumors as determined by vascular perfusion using fluorescein-labeled tomato lectin (green; n=4/5 for each group). The total area of the vascular bed was visualized by immunostaining for CD31 (red; n=4/5 for each group). The fraction perfused area was expressed as perfused area divided by total vascular area for each field. (d) Apoptotic index of D2F2/E2 tumor cells as determined by immunostaining for activated caspase-3 (n=4/5 for each group). *P<0.05, **P<0.01; Student’s t-test.

Agile Pulse In Vivo System
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