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Tech-Trends Application Note Volume 4, Series 6

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.

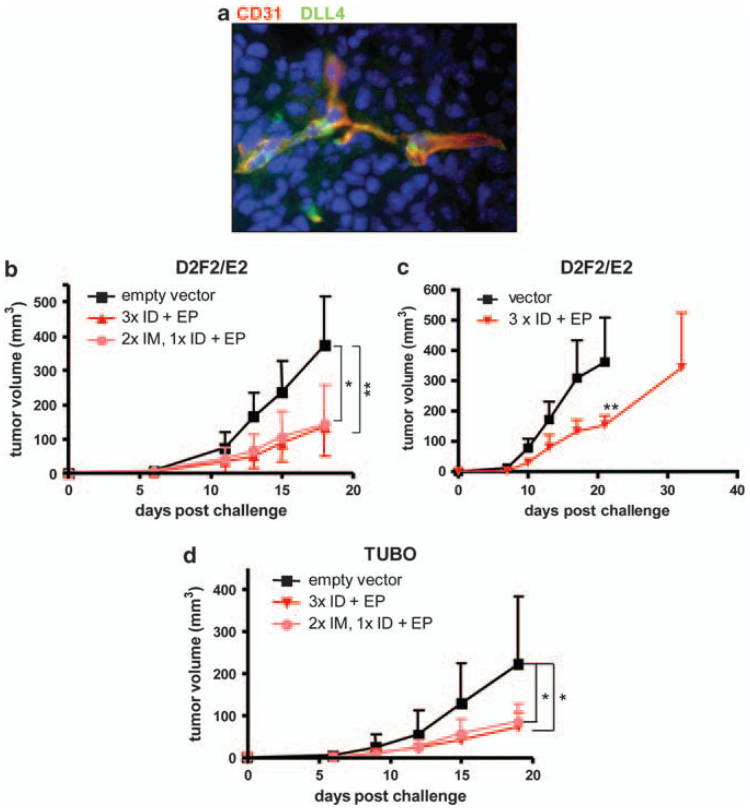

Fig. 2 DNA vaccination against DLL4 results in suppression of tumor growth in BALB/c mice. (a) Expression of DLL4 in D2F2/ E2 tumor endothelial cells was assessed by immunofluorescent staining for the endothelial markers, CD31 (red) and DLL4 (green). Cell nuclei were counterstained by DAPI (blue). Growth of orthotopically implanted D2F2/E2 (b and c) or TUBO (d) mammary carcinomas in BALB/c mice following immunization with empty vector or with DLL4 plasmid DNA according to protocol 1 (3IDþEP) or protocol 2 (2IM, 1IDþEP; n¼8 for all groups). The data shown in (b and d) are representative of three independent experiments. *Po0.05, **Po0.01; Student's t-test.

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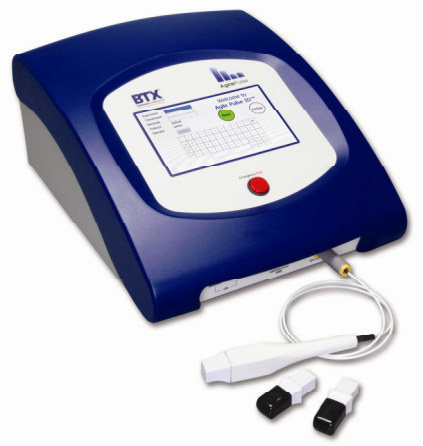
METHODS

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



Agile Pulse In Vivo System
Catalog 47-0400N

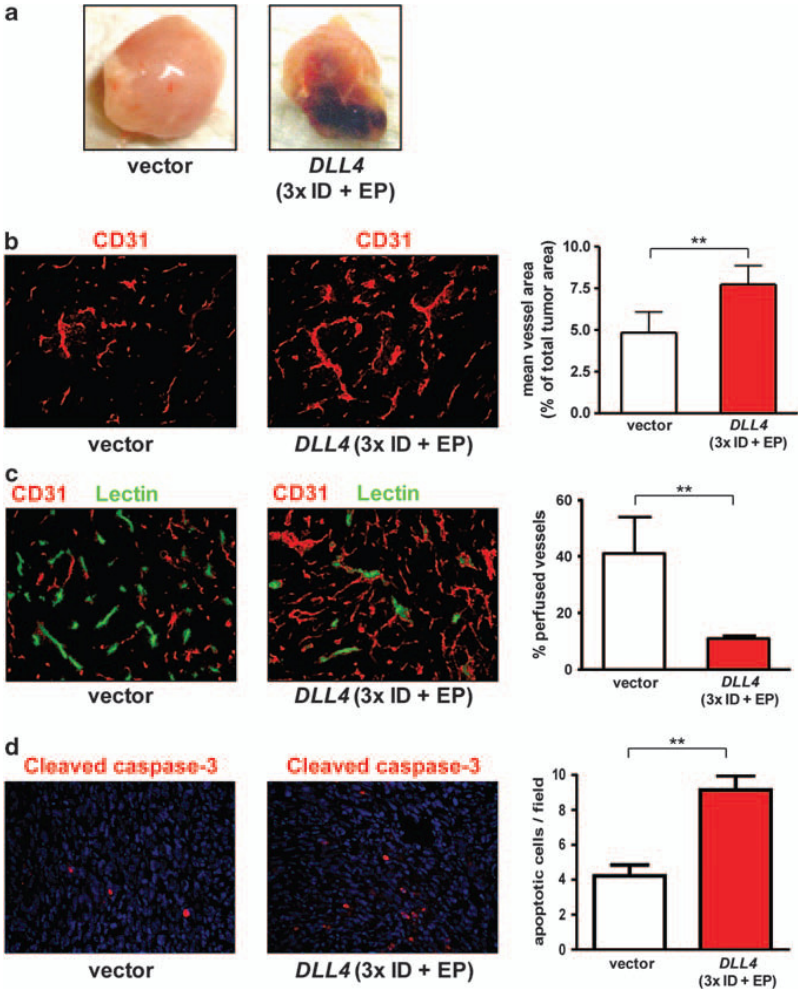


Fig. 3 Targeting DLL4 by vaccination results in excessive formation of nonfunctional blood vessels. (a) Visual inspection of excised D2F2/E2 tumors revealed overt thrombosis in tumors from DLL4-vaccinated mice. (b) Blood vessel density of D2F2/E2 tumors visualized by immunostaining for the endothelial cell marker CD31 (red; n¼5 for each group). (c) Perfused blood vessels of D2F2/E2 tumors as determined by vascular perfusion using fluorescein-labeled tomato lectin (green; n¼5 for each group). The total area of the vascular bed was visualized by immunostaining for CD31 (red; n¼5 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 (n¼5 for each group). *Po0.05, **Po0.01; Student's t-test.

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