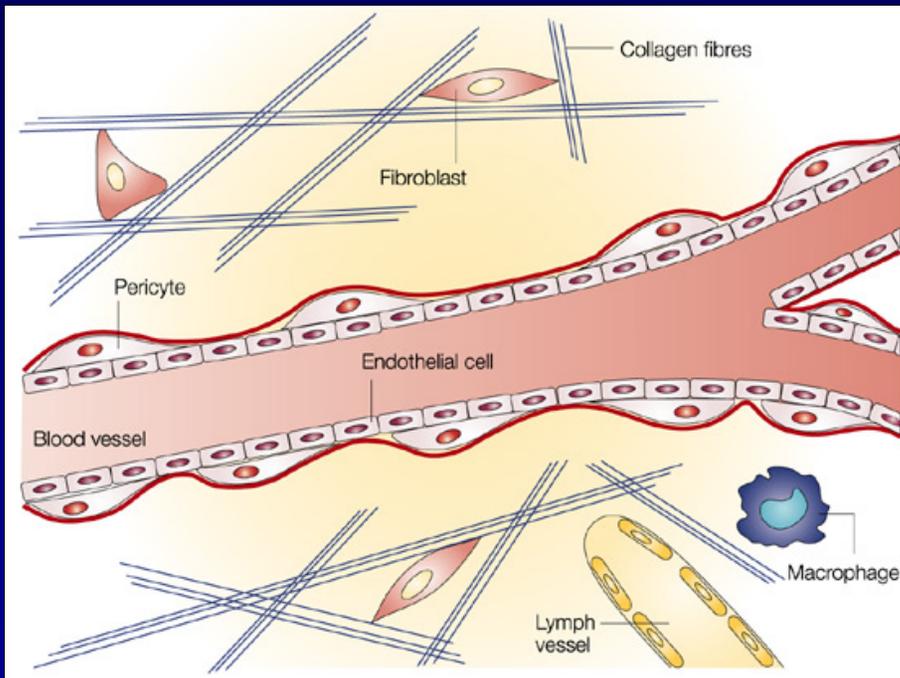


A DNA Vaccine Against Tumor Stromal Antigen FAP Boosts Chemotherapy

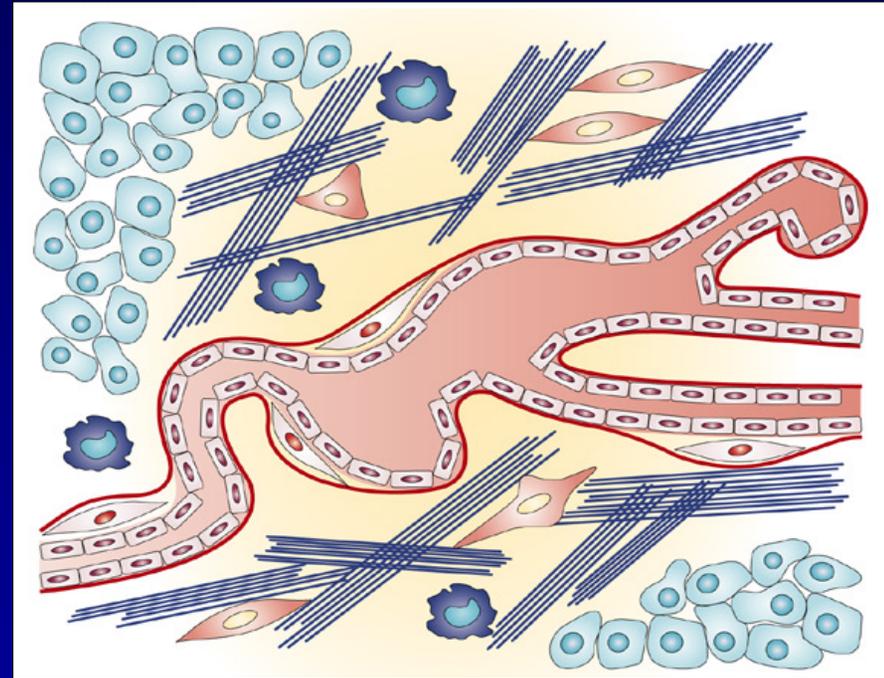


The Tumor Stroma

normal tissue



tumor tissue



Heldin *et al.* High Interstitial Fluid Pressure-An Obstacle in Cancer Therapy. *Nat. Rev. Cancer.* 4, 806-813. 2004.

The Tumor Stroma as a Target for Immunotherapy

Tumor-associated fibroblasts (TAFs) in the stroma are key regulators of tumorigenesis and invasion.

TAFs are the primary source of collagen type I which contributes to decreased drug uptake by tumors and plays a significant role in regulating tumor sensitivity to chemotherapeutics.

Fibroblasts in the tumor stroma synthesize fibroblast activation protein (FAP), a type II transmembrane protein functioning as a serine protease which is selectively overexpressed in over 90% of stromal fibroblasts associated with colon, breast and lung carcinoma.

Tumor-stromal expression and organization of collagen type I, which is mainly produced by fibroblasts, is inversely correlated with intratumoral uptake of chemotherapeutic agents, substantiating the involvement of the stromal compartment in resistance to chemotherapy.

Advantages of Tumor-Stromal Antigen FAP as a Target for T Cell-Mediated Cancer Immunotherapy

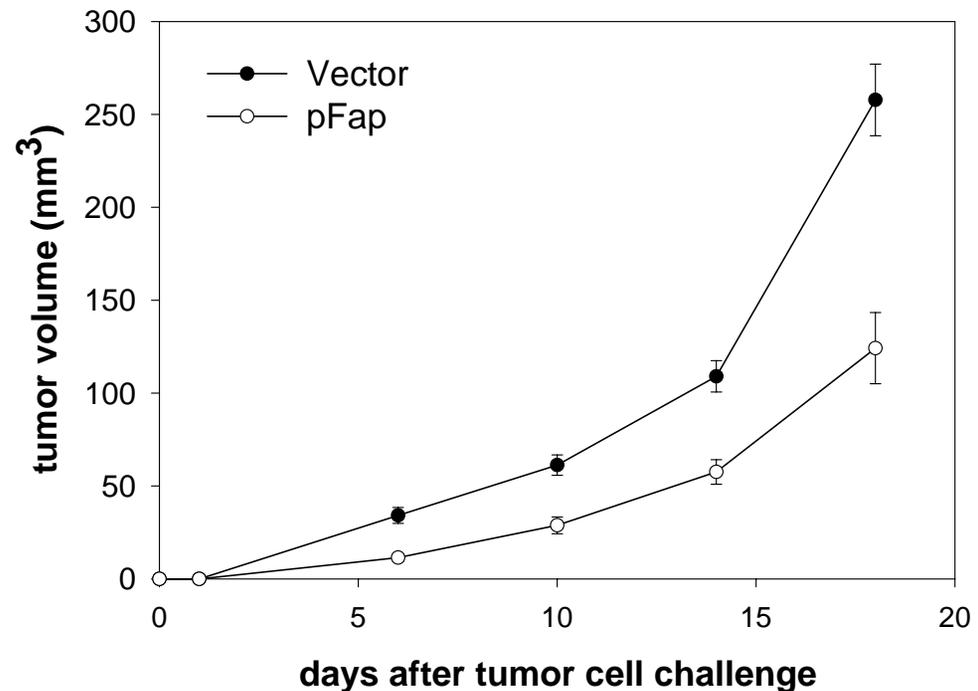
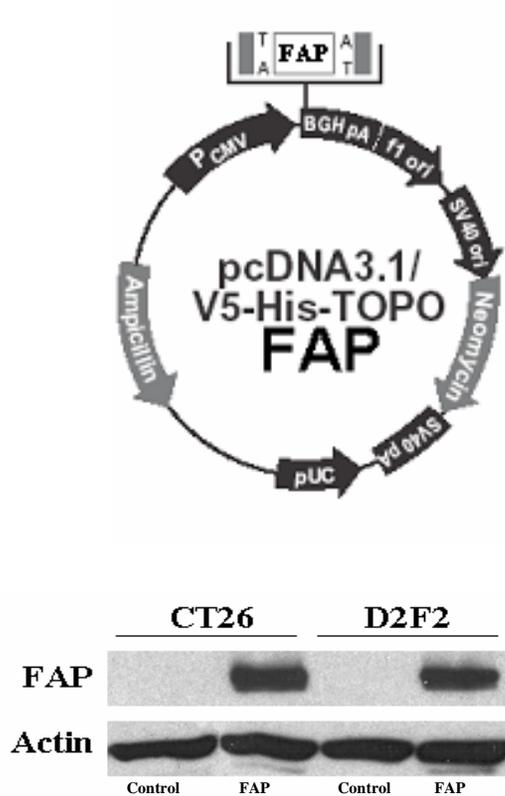
This approach has several advantages over therapies directed against antigens solely expressed by tumor cells.

First, antigen presentation by stromal fibroblasts to the T cell receptor complex is not impaired by down-regulated MHC class I antigen expression, as is frequently the case in human tumor cells.

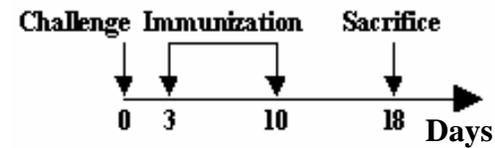
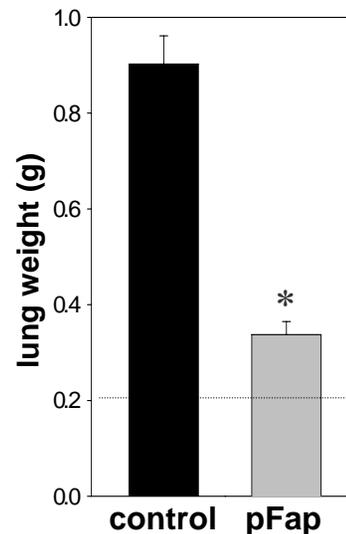
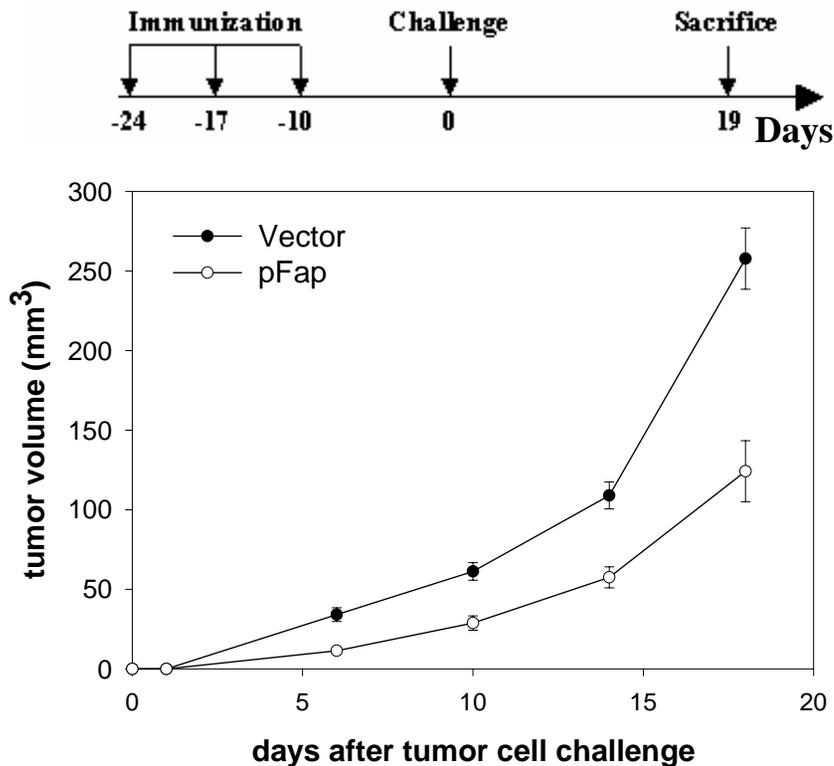
Second, tumor cells often become increasingly resistant to T cell-mediated killing due to defects in apoptosis signaling pathways, upregulation of antiapoptotic proteins, or immunosuppressive effects against CTLs.

Third, targeting FAP, specifically overexpressed in fibroblasts in over 90% of colon, breast and lung carcinomas allows for the treatment of several different malignancies in contrast to therapies involving antigens expressed solely by specific tumor types.

Cloning of FAP and Primary Tumor Growth of D2F2 Breast Carcinoma Cells



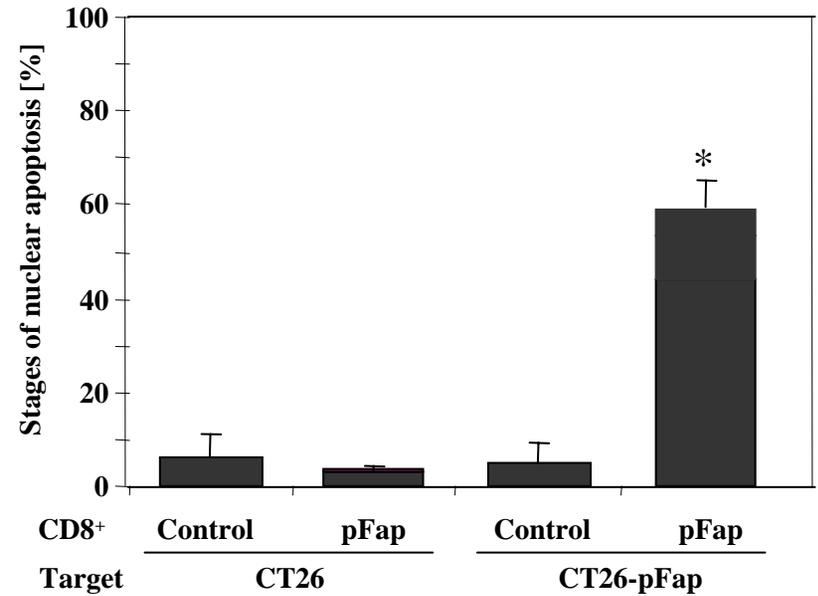
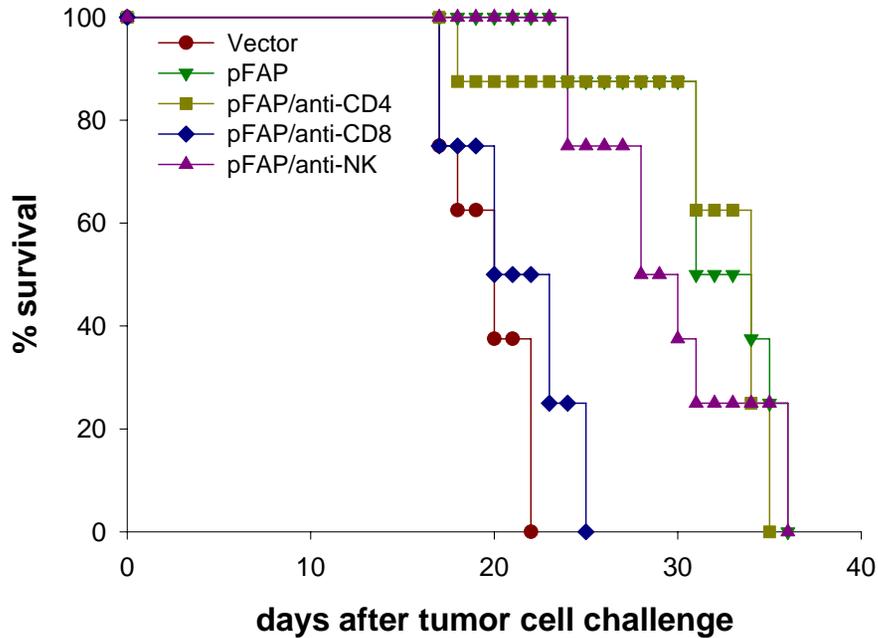
FAP Vaccine Suppresses Primary Tumor Growth and Metastases in a CT26 Colon Carcinoma Model



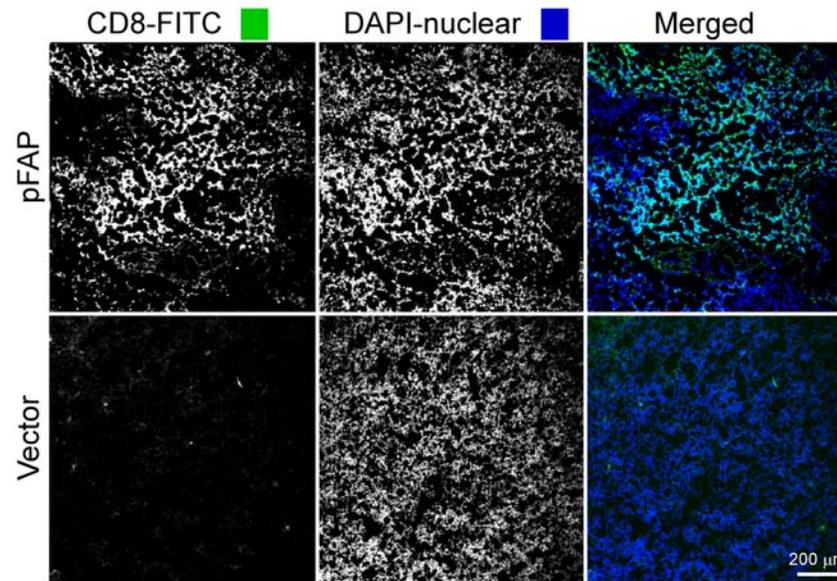
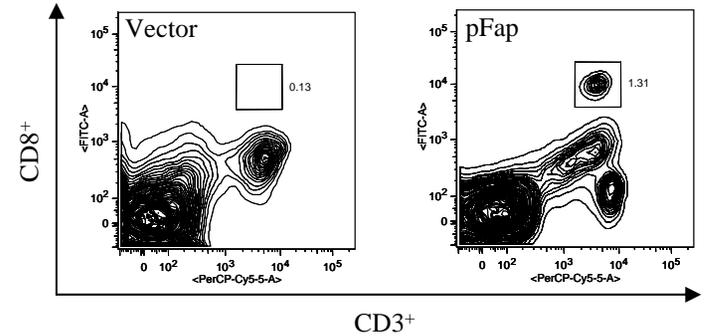
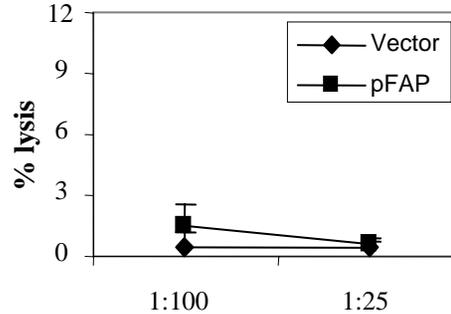
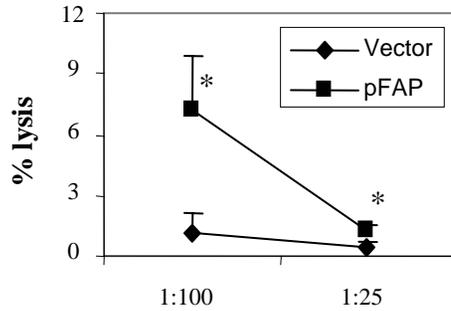
	Metastasis scores ^o
PBS	3, 3, 3, 3, 3, 3, 2, 2
pFap	2, 2, 2, 1, 1, 1, 1, 1

^o Percentage of surface coverage:
 0 = 0 %, 1 ≤ 20 %, 2 = 20-50%
 3 ≥ 50 %

Anti-Tumor Effect is Mediated by CD8⁺ T Lymphocytes (I)



Anti-Tumor Effect is Mediated by CD8⁺ T Lymphocytes (II)



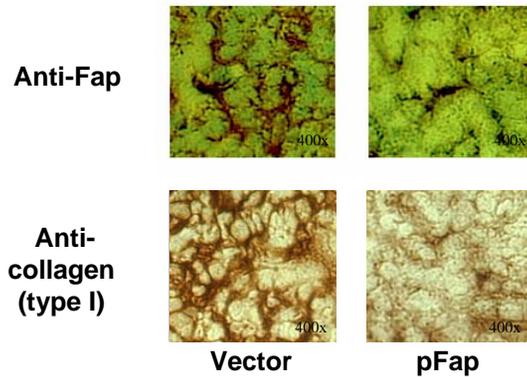
FAP Vaccine Improves Chemotherapy ?

- DNA vaccine against FAP moderately suppresses breast and colon carcinoma growth
- Fibroblasts produce collagen type I, which inversely correlates with intratumoral uptake of chemotherapeutic agent
- Tumor associated fibroblasts mediate chemoresistance via induction of anti-apoptotic pathways in tumor cells

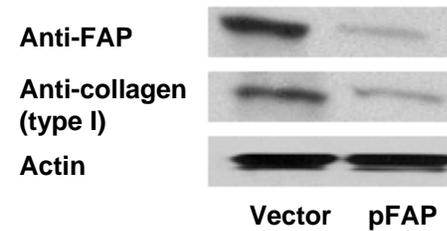
Hypothesis: FAP vaccine improves anti-tumor effects of chemotherapy

Fap Vaccine Increases Intra-Tumoral Uptake of Fluorescein, Albumin and 5-FU

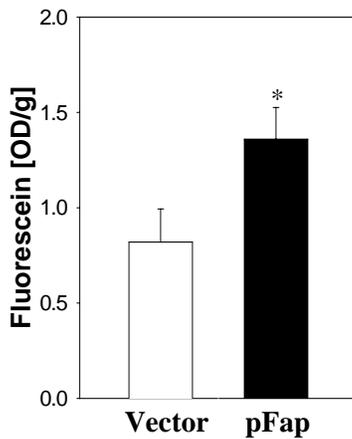
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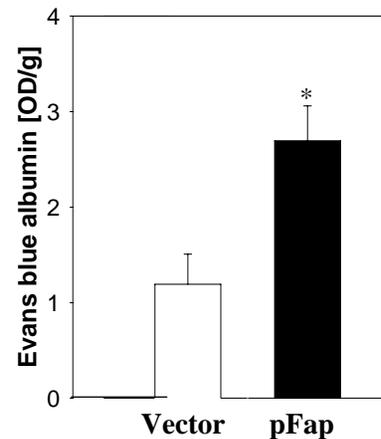
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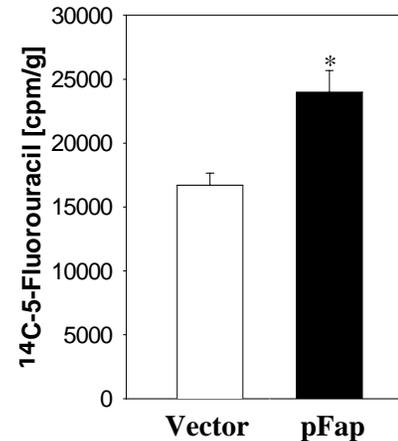
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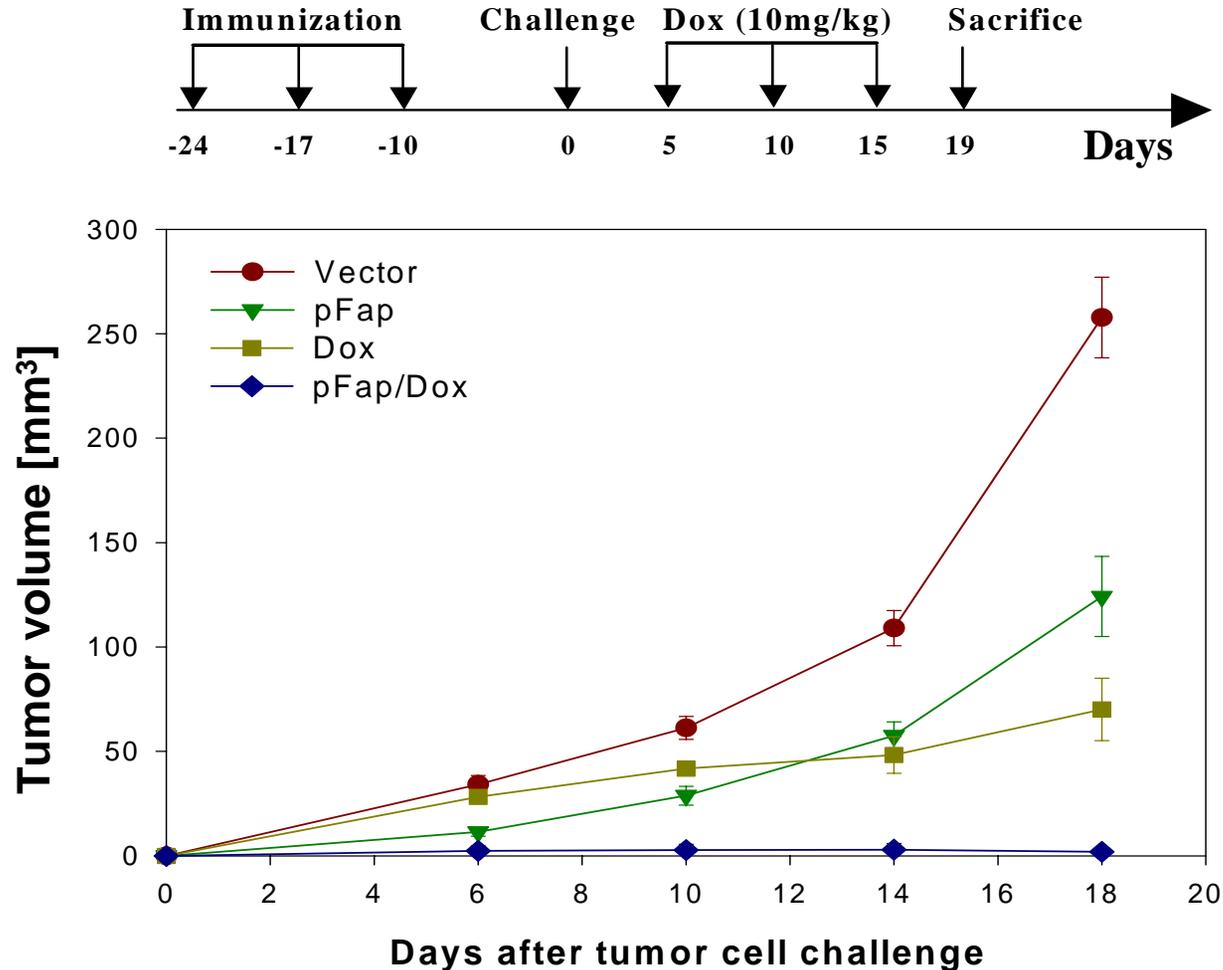
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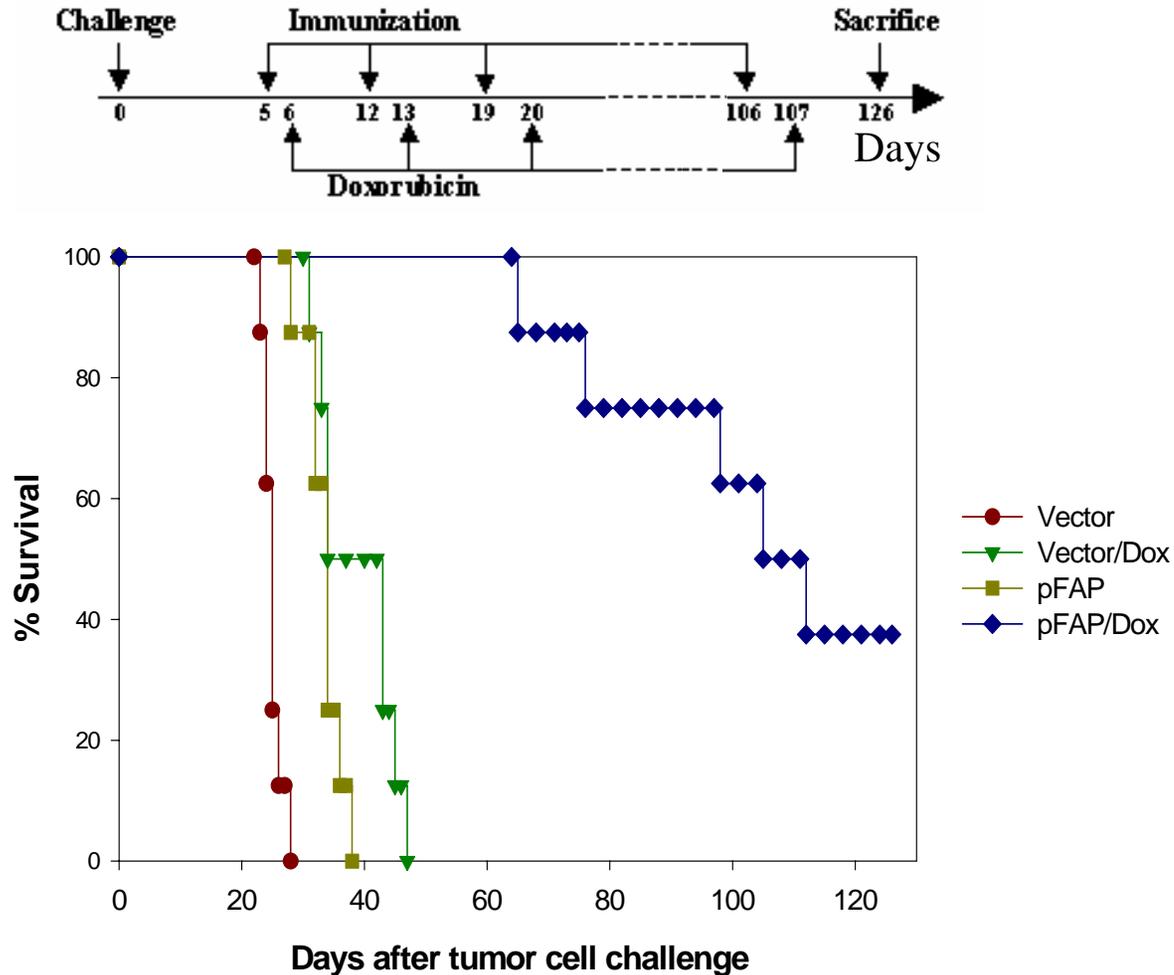
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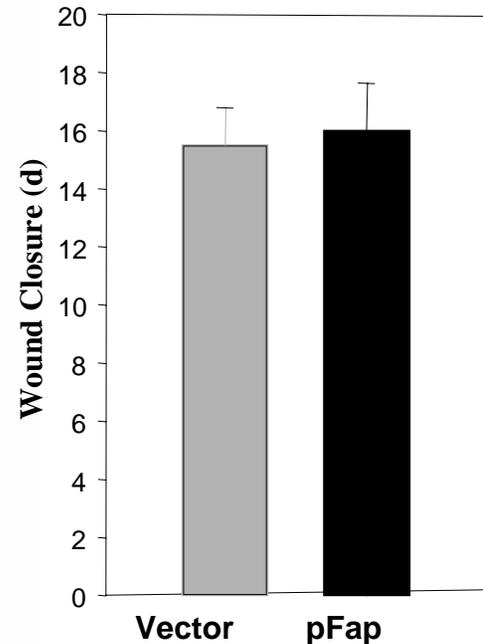
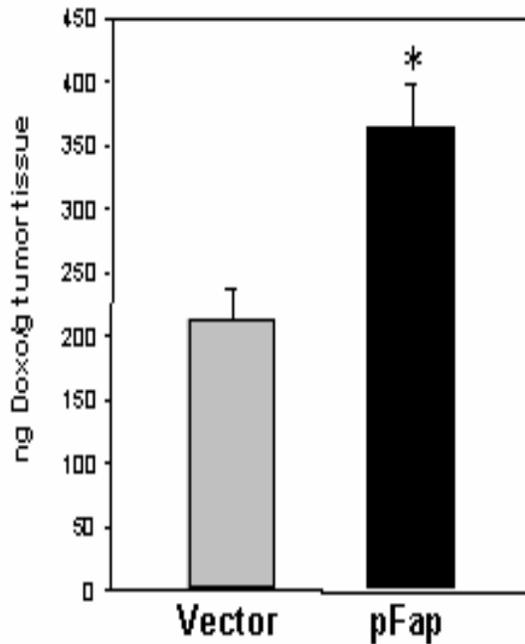
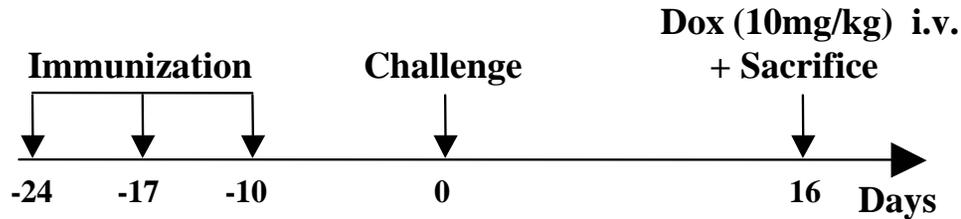
Combinational Therapy Inhibits Primary Tumor Growth in a D2F2 Breast Carcinoma Model



Combinational Therapy Prolongs Life Span in a Therapeutic D2F2 Breast Cancer Model



FAP Vaccine Increases Intratumoral Uptake of Doxorubicin in D2F2 Breast Cancer Model



Conclusions

- **An oral DNA vaccine encoding fibroblast activation protein (FAP), overexpressed on fibroblasts in the tumor stroma, induced a CD8+ T cell response which killed tumor-associated fibroblasts and suppressed primary growth and metastasis of multi-drug resistant murine colon and breast carcinoma.**
- **Tumor tissue of FAP-vaccinated mice revealed markedly decreased collagen type I expression and up to 70% greater uptake of chemotherapeutic drugs.**
- **pFAP-vaccinated mice treated with chemotherapy, showed a 3-fold prolongation in lifespan and marked suppression of tumor growth with 50% of animals completely rejecting a tumor cell challenge.**
- **This strategy opens a new venue for the combination of immuno-and chemotherapies.**



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Characterization of the FAP construct and chemoresistant tumor cell lines

