

Quantification of Tumor Burden in Patient-Derived Orthotopic Models of Ovarian Cancer by Fluorescent and Bioluminescent 3-D Imaging

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Short Abstract – Ovarian cancer is often diagnosed at a late stage when cancer cells have disseminated throughout the peritoneal cavity and formed tumors on multiple organs. We have established orthotopic models of disseminated ovarian cancer from patient-derived cancer spheroids to test novel therapies in the context of the peritoneal microenvironment. Fluorescent and bioluminescent imaging and 3-D reconstruction allows us to estimate changes in tumor burden in these patient-derived orthotopic models. The pan-ErbB inhibitor, afatinib, alone or in combination with paclitaxel, was tested in these models.

Keywords — ovarian cancer, *in vivo* imaging, patient-derived xenografts, afatinib

I. BACKGROUND

Preclinical studies in mouse models of cancer are important for initial validation of novel therapies. It has become increasingly apparent that the tumor microenvironment plays an important role in the growth and metastasis of tumors as well as in the response of cancer cells to treatment¹. Therefore, orthotopic tumors growing in their preferred microenvironment may best reflect human cancer. We have used an orthotopic model of disseminated ovarian cancer to demonstrate that ovarian cancer tumors exhibit different morphology depending on the site of metastasis². These studies were used to parameterize a 3-D cellular Potts model and examine drug penetration in ovarian tumors³. To better represent tumor heterogeneity, we have now developed orthotopic patient-derived xenograft models. The development of new techniques in *in vivo* imaging are improving the estimation of tumor burden in these models. Here, fluorescent and bioluminescence imaging was used to assess changes in tumor burden during treatment with a pan-ErbB receptor inhibitor compared to chemotherapy or combination therapy.

II. METHODS

Patient-derived xenograft (PDX) models were established by isolating cancer spheroids from fresh patient ascites fluid obtained at the time of surgery. Spheroids were injected into the peritoneal cavity (IP) of NOD-*scid* gamma (NSG)

immunocompromised mice to model disseminated ovarian cancer. Five PDX lines have been established. PDX lines are maintained passaging them into new mice.

Tumor burden was assessed in non-transduced PDX models using a human-specific anti-HLA-ABC antibody conjugated to the near-infrared dye CF750. For bioluminescence imaging, cancer cells from PDX models were cultured as spheroids and transduced with an R-luciferase reporter carrying puromycin resistance. After expansion in a mouse, transduced spheroids were selected for puromycin resistance in culture before passaging in mice.

Five mice/group were inoculated with PDX cancer spheroids and treated with paclitaxel, afatinib or in combination for three weeks. Tumors were imaged pretreatment and every week during treatment. Tumor burden at necropsy was compared to *in vivo* imaging. Imaging was performed on the IVIS Spectrum *in vivo* imaging system. Fluorescent imaging tomography (FLIT) or diffuse luminescence tomography (DLIT) were used to construct 3-D images of tumor positions. DLIT images can be calibrated to the luminescence rate (photons/cell/sec) to estimate the number of cells in a given tumor.

III. RESULTS

Peritoneal tumors were visible one to two weeks post-inoculation using either fluorescent or bioluminescent probes. Treatment with afatinib, paclitaxel, or the combination reduced tumor burden over time with the greatest affect seen in combination-treated animals. Imaging of control mice at later time points was complicated by the development of ascites fluid that appears to dampen or dilute the fluorescent signal. In this case, imaging at necropsy revealed more tumors than could be seen in the live image.

IV. Conclusion

The use of 3-D *in vivo* imaging techniques with fluorescent or bioluminescent reporters provides a more quantitative way to assess tumor burden in orthotopic models of cancer. These techniques may provide unique data sets that could be used to parametrize multiscale models of tumor growth and response to therapies.

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