SUPPLEMENTARY RESULTS AND DISCUSSION

Tumor grafts represent the heterogeneity of breast cancer. Breast cancer is a heterogenous disease with respect to pathology, histology, mutations, gene expression, metastasis profiles, and response to therapy. As a result, breast cancer may be more accurately classified as a collection of related diseases, rather than as one disease. Thus, an important feature of the tumor grafts described herein is the retention of unique gene expression profiles and DNA copy number variants found in the original tumor samples. This manifests in maintenance of the molecular subtypes of the breast cancers, which will be important for modeling the biology of various types of breast cancer or for drug development toward specific breast tumor subtypes.

Our approach was to use genomic information, in the form of gene expression profiles and DNA copy number variants, to assess the relative similarities and differences between the original tumor specimens and tumor grafts. We noted that HCI-008 gave discrepant results between two pleural effusions isolated directly from this individual, switching from Basal-like in the first pleural effusion sample to Luminal B in the second sample. According to gene expression microarray data, the two samples from this individual did not represent each other or other subtypes. This unusual scenario might reflect the heterogeneity of the original pleural effusion sample and/or effects of treatment. We favor the latter scenario because, in fact, this sample yielded two different tumor types upon transplantation into multiple mice: breast cancer and $CD45^+$ human lymphoma (**Supplementary Fig. 32**). Serial propagation of the breast cancer resulted in a homogeneous $ER^-PR^-HER2^+$ breast tumor line (**Supplementary Fig. 10**); the lymphoma line was not further propagated. Unfortunately, this patient died shortly after obtaining the samples that gave rise to the tumor grafts, and lymphoma was not diagnosed by the

time of her death. We therefore concluded that the "switch" in molecular subtype classification is most likely a result of heterogeneity in the tumor sample, potentially confounded by a lymphoma.

The only other discrepancy observed between original human tumors and tumor grafts was in case HCI-009: an individual whose ascites fluid contained tumor cells that were initially classified as HER2-enriched, but subsequently classified as Luminal B from the tumor graft. Three serial transplants from this subject all classified as Luminal B, suggesting that the transplants remained stable. All samples from HCI-009 appeared to be Luminal B by unsupervised clustering analysis as well. Conversely, for case HCI-012 the clustering showed a HER2-enriched tumor subtype for both the original human tumor and tumor graft using unsupervised clustering, but both were classified as Luminal B by the supervised PAM50 algorithm. Together, these data indicate that while there were occasional "borderline" cases in which the subtype was difficult to determine even from primary specimens, the molecular features of tumor grafts strongly reflected those of the original tumors.

Common copy number variations occur across multiple tumors and tumor grafts. There were copy number variations in certain regions that were consistent across most tumors and tumor grafts (**Fig. 4b**). Examples include large amplifications on chromosomes 8 and 1q, which were found in all samples except HCI-008 ($HER2^+$ inflammatory breast cancer). Large gains on chromosome 7 were seen in all except the two $ER^-PR^-HER2^+$ tumors, and the expected gains in chromosome 17q were seen in all of the $HER2^+$ samples except HCI-012, which was from a relapsed tumor refractory to herceptin (**Supplementary Table 1)**.

In several cases, we were also able to glean information about how stable the tumor grafts remained with serial passage in mice, or after a relapse of disease (**Supplementary Fig. 26**). Although our approach was not an attempt to thoroughly characterize the tumor graft bank, which is beyond the scope of this report, these data will contribute to the utility of these tumor grafts as a technical resource for future research and drug development.

Degree of immunodeficiency effect growth of some tumor grafts. Multiple groups[1,2] have noted the importance of using mice that are more severely immuno-compromised than NOD/SCID mice for engraftment of primary human tumors (i.e. the double mutant NOD/SCID;IL2Rγ-/- (NSG) mice, which lack NK cells as well as mature lymphocytes[3]). Although we began our study when NSG mice were not as readily available, and have continued using NOD/SCID mice for the sake of consistency, we have tested the growth of several lines in NSG mice. We have found that certain tumors (i.e. HCI-004 and HCI-008) grow faster in NSG mice than in NOD/SCID, and others grow equally well in both (i.e. HCI-012) (**Supplementary Table 1**); no significant differences in metastasis have been noted thus far. It is important to note, however, that our studies were not carried out at limiting dilution, and that larger differences in tumor behavior in different mouse strains might be apparent under more stringent conditions. These data do suggest that, as previously reported[4], tumor progression depends on interactions between the immune system and tumor, which can significantly affect tumor behavior.

Together, our findings indicate that tumor grafts are excellent models for human breast cancer. Because of their high potential for clinical relevance, and their ease of use once

established, tumor grafts should provide ample opportunities to significantly impact breast cancer research and therapy.

REFERENCES FOR SUPPLEMENTAL RESULTS AND DISCUSSION

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