Fusion transcript discovery in formalin-fixed paraffin-embedded human breast cancer tissues and its relation to tumor progression.

Author(s): Yan Ma, Ranjana Ambannavar, James Stephans, Jennie Jeong, Andrew Dei Rossi, John Morlan, Mei-Lan Liu, Samuel Levy, Joffre Baker, Dominick Sinicropi, Kunbin Qu; Genomic Health, Inc., Redwood City, CA

Background: While several recently discovered gene fusions already play an important role in personalized cancer treatment, many cancer gene fusions remain to be discovered. Next generation sequencing has enabled identification of many rare gene fusion events in fresh or frozen solid tumors. There is a need to detect gene fusions in transcriptomes of formalin-fixed paraffin-embedded (FFPE) tumor tissue, for which there is long-term clinical outcome data. We therefore sought to develop bioinformatics methods to detect fusion transcripts in FFPE tissue and to characterize their association with clinical outcomes. **Methods:** RNA sequencing libraries were created and sequenced from tumor biopsy tissues (Plos One 2012 7(7): e40092) of two ER+ breast cancer cohorts consisting of 136 and 77 patients, for which clinical outcomes were available. The fusion junctions were nominated by the RNA-seq aligner GSNAP and further filtered to consider discontinuous expression patterns at exon/intron levels. **Results:** A total of 108 candidate fusion transcripts were detected and RT-PCR assays confirmed 89% of the top ranking fusion transcript candidates. The majority (82%) of identified fusion gene partners are listed in the COSMIC database of known cancer sequence variations. Of note, several patients expressed multiple fusion transcripts that are significantly associated with tumor progression ($P<0.001$), including genes associated with cell proliferation and cellular metabolism. Furthermore, these patients also harbored inter-chromosomal gene fusions. It is noteworthy that several gene fusions were present in multiple patients. In one of these recurrent fusions the estrogen receptor gene acts as the fusion pair donor. **Conclusions:** Novel bioinformatics approaches developed here demonstrate the ability to detect fusion transcripts as biomarkers from archival FFPE tissues that associate with breast cancer progression. Some gene fusions were common in multiple patients and deserved further study.