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Genomic Analysis of Advanced Breast Cancer Using Two Types of Next Generation Sequencing

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Introduction

The aim of this study is to characterize the genomic alterations of advance stage breast cancer using next generation sequencing (NGS) to identify pathways that may be commonly altered in advance stage breast cancer.

Materials and Methods

Twenty patients with stage 4 invasive ductal breast cancer who had received NGS on resection tissue and NGS on circulating tumor DNA (CT-DNA) at least once during their treatment course were identified. The patients were divided up by immunoprofile and the genomic results were reviewed.

The genomic analysis was not time standardized, but most patients had tissue NGS done on a mastectomy specimen after neoadjuvant chemotherapy. NGS on CT-DNA varied and was frequently done more than once on patients and often both before and after the resection. However, many patients had their initial diagnosis and some treatment elsewhere and were referred for further management, so CT-DNA NGS was done at the time of referral after treatment and resection.

The two commercially available NGS sequencing panels used were FoundationOne and Guardant Health. Both use targeted NGS to look for a panel of common cancer mutations. FoundationOne is a 236 gene panel plus 47 introns from 19 other genes done on formalin fixed paraffin embedded tissue. It uses massively parallel next generation sequencing to achieve a median depth of 500x. Guardant is done on circulating tumor DNA extracted from blood plasma using a quiagen circulating nucleic acid kit. It is a 68 gene panel with 29 genes completely covered. It has an average depth of approximately 5,000x. All of the genes on the Guardant Health panel are also covered on the FoundationOne panel. FoundationOne testing methods are commercially available and have been validated for sensitivity, specificity, and diagnostic accuracy in prior studies. Guardant health validation studies are forthcoming, but initial data also shows good sensitivity, specificity, and diagnostic accuracy.

The NGS results were sorted by alteration type and molecular pathway. Alteration type included mutations, amplifications, deletions, or rearrangements. The molecular pathways characterized included MTOR, p53, cell cycling, receptor tyrosine kinase/growth factor (RTK/GF), and BRCA.

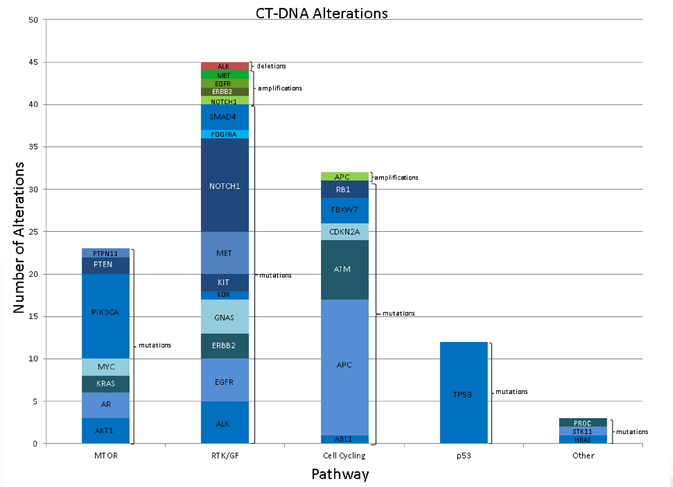

Figure 1.1 - Circulating Tumor DNA Alteration Graph: This graph shows the number of alterations detected in CT-DNA in each pathway. Each column represents a pathway. Each pathway is divided by the type of alteration (deletions, amplifications, and other alteration) and the specific gene altered in the appropriate proportion.

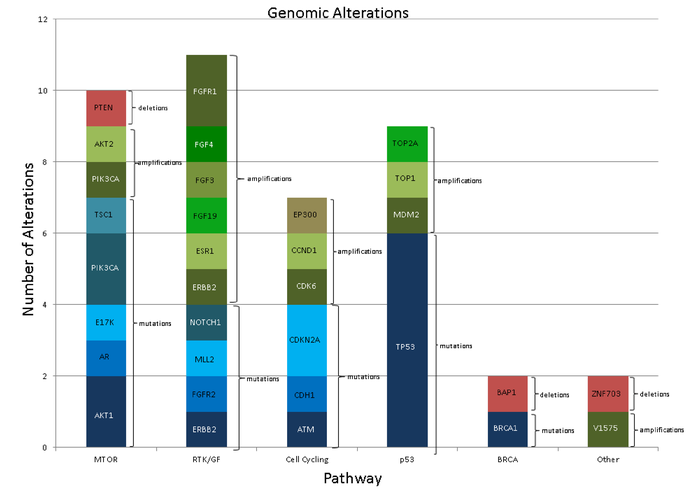

Figure 1.2 - Tissue Genomics Alteration Graph: This graph shows the number of alterations detected by tissue NGS in each pathway. Each column represents a pathway. Each pathway is divided by the type of alteration (deletions, amplifications, and other alteration) and the specific gene altered in the appropriate proportion.

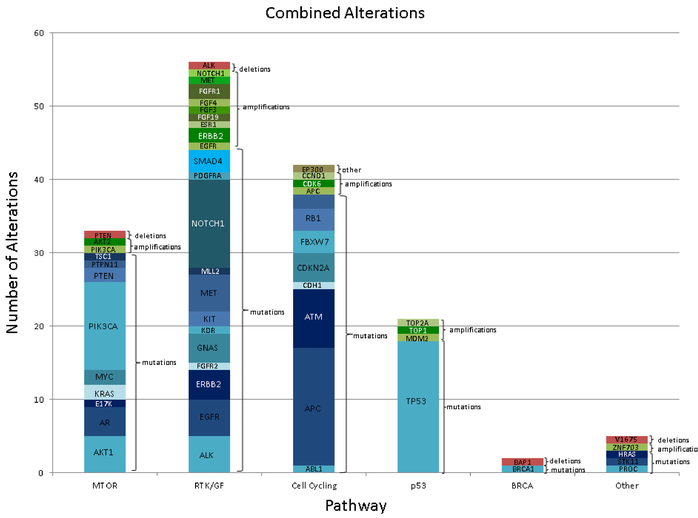

Figure 1.3 - Combined Methods Alteration Graph: This graph shows the number of alterations detected by either NGS method in each pathway. Each column represents a pathway. Each pathway is divided by the type of alteration (deletions, amplifications, and other alteration) and the specific gene altered in the appropriate proportion.

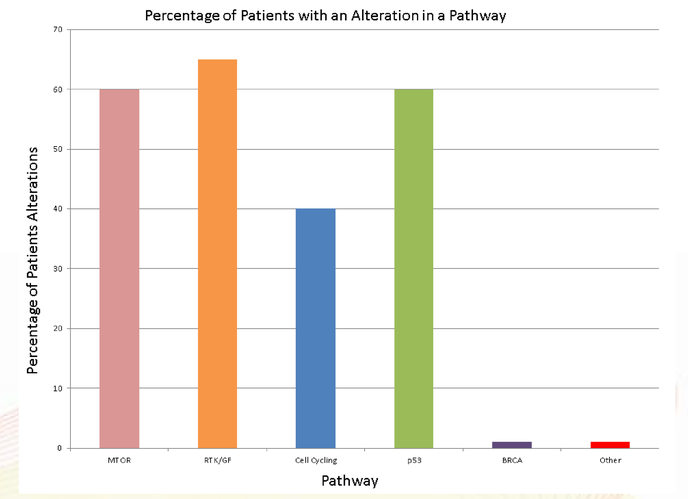

Figure 1.4 -Percentage of Alterations per Patient Graph: This graph shows the percentage of alterations in each pathway. Each column represents a pathway.

Results

A total of 115 alteration were found using CT-DNA (figure 1.1). Of those alterations, the most commonly altered pathway was the RTK/GF pathway which constituted almost 40% of the total number of alterations found using this method. Of those, mutations were most commonly seen in a variety of different genes but most prominently in NOTCH 1. The second most commonly altered pathway detected in CT-DNA was cell cycling which accounted for 29% of the alterations, almost all of which (97%) were mutations. Fifty two percent of the mutations in the cell cycling pathway were in the APC gene. The next most commonly altered pathway was the MTOR pathway which accounted for 20%, all of which were mutations. Forty three percent of these mutations were seen in PIK3CA. Ten percent of alterations were seen in the p53 pathway, all of which were mutations in TP53. Finally, the remainder of the alterations were seen in the other category and were entirely mutations.

Using tissue genomic analysis on resection specimens, a total of 41 alterations were found (figure 1.2). The most commonly altered pathway detected using this method was again the RTK/GF pathway, which accounted for 27% of the total alterations seen. Unlike in the CT-DNA analysis, however, most of these alterations (64%) were amplifications in various genes. The second most commonly altered pathway was the MTOR pathway, which accounted for 24% of the total alterations . Seventy percent of the alterations were mutations. Next most commonly altered was the p53 pathway which accounted for 22% of the total alteration, 67% of which were mutations in TP53. Seventeen percent of the alterations were in the cell cycling pathway which was broken down in to 57% mutations and 29% amplifications. Less commonly altered pathways included the BRCA pathway and the other category which each accounted for 5% of the alterations seen.

If both CT-DNA and tissue genomics alterations were analyzed together, a total of 156 unique alterations were detected (figure 1.3). Once again, RTK/GF accounted for the largest percentage of these alterations at 36%. Seventy nine percent of the alterations in RTK/GF were mutations, of which almost 30% were mutations in the NOTCH1 gene. The second most commonly altered pathway was the cell cycling pathway, accounting for 25%. Almost 90% of these alterations were mutations and 46% of these occurred in the APC gene. The next most commonly altered pathway was the MTOR pathway which accounted for 21% of the total alterations. Ninety one percent of the alterations seen were mutations, and 40% of them occurred in PIK3CA. P53 accounted for 14% of the total alterations detected using both methods, and 86% of these were mutations in the TP53 gene. Finally, less commonly altered pathways included the BRCA and other categories which accounted for 1% and 3% of the total alterations respectively.

If looked at in terms of what percentage of patients displayed an alteration in a given pathway, 65% of patients had an alteration in the RTK/GF pathway, 60% had an alteration in MTOR, 60% had an alterations in p53, 40% had an alteration in cell cycling, 1% had an alteration in BRCA, and 1% had an alteration in the other category (figure 1.4).

Conclusion

Stage 4 breast cancer is a molecularly heterogeneous group of cancers. with a variety of genomic alterations seen. Most notably, alterations occurred in the RTK/GF pathway. However, caution should be used in interpreting this since it this category includes the largest number of genes. The cell cycling pathway is similar and also was prominently altered in this cohort. Finally, MTOR and p53 are both important pathways for advance stage breast cancer and displayed both a large number of overall alterations as well as a significant percentage of patients (60%) displaying alterations in them. As genomic analysis continues and targeted therapies increase, trends and commonalities among cancer types will become increasingly important. This study identified several pathways including RTK/GF, p53, MTOR, and cell cycling that are commonly altered and may present targets for future therapies.