Mutated Pathways as a Guide to Adjuvant Therapy Treatments for Breast Cancer

Yang Liu, Zhenjun Hu, and Charles DeLisi

Abstract

Adjuvant therapy following breast cancer surgery generally consists of either a course of chemotherapy, if the cancer lacks hormone receptors, or a course of hormonal therapy, otherwise. Here, we report a correlation between adjuvant strategy and mutated pathway patterns. In particular, we find that for breast cancer patients, pathways enriched in nonsynonymous mutations in the chemotherapy group are distinct from those of the hormonal therapy group. We apply a recently developed method that identifies collaborative pathway groups for hormone and chemotherapy patients. A collaborative group of pathways is one in which each member is altered in the same—generally large—number of samples. In particular, we find the following: (i) a chemotherapy group consisting of three pathways and a hormone therapy group consisting of 20, the members of the two groups being mutually exclusive; (ii) each group is highly enriched in breast cancer drivers; and (iii) the pathway groups are correlates of subtype-based therapeutic recommendations. These results suggest that patient profiling using these pathway groups can potentially enable the development of personalized treatment plans that may be more accurate and specific than those currently available. Mol Cancer Ther; 15(1); 184–9. ©2015 AACR.

Introduction

Breast cancer is a highly heterogeneous disease consisting of distinct molecular subtypes having different prognostic and therapeutic responses (1). Of interest to this article is that surgery is often followed by adjuvant therapy to diminish the chance of recurrence. Chemotherapy is used to stop the growth of cancer cells by either killing them or otherwise halting division (2); hormone therapy lowers estrogen levels or blocks its action (3). The choice between the two is usually made based on the levels of (4, 5) estrogen receptor (ER), progesterone receptor (PR), and HER2 (6). In general (4), Luminal-A is treated with hormone therapy; luminal B is often treated with chemotherapy, but occasionally with hormone therapy; and HER2 and basal-like subtypes are treated with chemotherapy.

Until now the choice of therapy has been guided by the levels of RNA transcripts of the three hormone receptor genes. Our understanding of cancer biology (7) and the state of computational science (8–15) has, however, now reached a point where a much fuller profile of the cancer cell can be used to guide the choice of therapy.

The development of cancer is a complex multistep process that involves accumulation of multiple mutations that lead to dysfunction of cell signaling pathways responsible for cell growth and cell fate (16). In particular, mutations are not uniformly distributed across different cellular functions, but tend to cluster in a relatively well defined set of physiologically relevant pathways (17). Consequently, it is now generally accepted that causal mechanisms underlying transformation generally reflect the behavior of functionally coupled sets of genes (18–20). An analysis of mutual heterogeneity in the context of cellular signaling and regulatory pathways can therefore add to our understanding of cancer progression and its modulation by different therapeutic strategies (16).

We identified sets of mutated pathways that are found in a high percentage of TCGA (21) breast cancer samples derived from patients to whom adjuvant therapy had been, or was being, administered. We refer to these pathways as collaborative because they are mutated in a high percentage of the same samples, as opposed to pathways that are altered in different but overlapping sets of samples. This analysis was performed using a recently developed method, Mutational Driver Pathway Collaboration (MUDPAC; ref. 20). Our goal was to determine whether different therapy groups—the chemotherapy group (CT) and the hormone therapy group (HT)—have distinguishing sets of mutated pathways, and if they do, to determine the composition of the two groups. The central result of this article is that we found strong correlation between altered processes (collaborative pathway groups) and therapy group. This suggests a useful addition to our knowledge of cancer subtypes and provides a sound molecular basis for subtype recommendations, with perhaps some moderate shifts in recommendations. These observations could have important implications for cancer biology and therapy, which we discuss later. A flowchart of our method can be seen in Fig. 1.

Materials and Methods

Somatic mutation data

Breast cancer tissue somatic mutations (.maf file) were downloaded from TCGA on March 2013. All mutations in .maf file are sequenced and annotated before any systemic treatment and therapy (22).
mutually exclusive to each other. We hope mutated pathway groups could be considered as a signature in the future to stratify subtypes and optimize therapeutic treatments.

Tumor sample selection

Tissue sampling is carried out by the TCGA project; no further tissue sampling is performed in this study. Sample subtype information was obtained from the supplementary table of ref. 21 where PAM50 is used to stratify subtypes based on mRNA data. The therapy treatment and drug information of each patient was downloaded from breast cancer TCGA clinical data released in July 2013. Only chemotherapy and hormone therapy patients are included in this study, which are the only adjuvant treatments with a sufficient number of samples in TCGA (>50). Cooperative pathways are identified using samples from patients who have been treated by chemotherapy or hormone therapy, but not both. After filtering, 56 chemotherapy samples remained (34 basal-like, 10 HER2⁺, 9 Luminal-A, 3 Luminal-B), with 3,971 mutations in 3,186 genes, and 51 hormone therapy samples remained (1 basal-like, 0 HER2⁺, 40 Luminal-A, 10 Luminal-B), with 2,049 mutations in 2,049 genes.

Pathway data

A total of 269 pathways (.xml and .gene files) were downloaded from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database released on June 2013; 200 of them are used in this study after excluding 3 global metabolic pathways and 66 human disease pathways.

MUDPAC

MUDPAC is a method that identifies mutated collaborative pathway groups, i.e., pathways that are altered in a high percentage of the same samples of a certain cancer or cancer subtype (20). It consists of two steps. The first step is an identification of pathways showing statistically significant differences between nonsynonymous mutation group and synonymous mutation group, based on some characteristics of mutations, such as mutation frequency, functional mutation score, mutual exclusivity, and network topology. The second step uses a greedy search for collaborative pathways. Two more criteria are considered when selecting a new pathway into collaborative set. First, the newly selected pathway along with all pathways already in collaborative set should have a maximal coverage rate (MCR) higher than the highest mutation rate of genes in this new pathway by a given threshold (3% in this study). Second, permutation is used to test whether MCR of this new pathway is significantly higher than a background MCR (P < 0.05 in this study).

Each of the missense mutations in .maf file is assigned a functional mutation score by MutationAssessor (23) based on hg19 reference genome. Because MutationAssessor can only evaluate missense polymorphisms, the functional scores for the remaining mutations are assessed following the same criteria in MUDPAC: the highest score that can be calculated using MutationAssessor is assigned to all indels, nonsense mutations, and splice site mutations; the lowest score from MutationAssessor is allocated to synonymous mutations; the average score of all remaining mutations are assessed following the same criteria in MutationAssessor.

MuSiC (24) is used to calculate the total number of bases for each gene having available alignment data, as part of the input required by MUDPAC. MuSiC uses Broad Institute’s analysis infrastructure Firehose to count bases with sufficient coverage of each gene from the given wiggle track format file. Wiggle files contain dense, continuous data, such as GC percent, probability scores, and transcriptome data, and were downloaded from Broad Institute Firehose webpage on August 2012. The thresholds for sufficient coverage are at least 8-fold read depth in normal tissue, and at least 14-fold read depth in cancer tissue.

Statistical analysis

The Fisher exact test is used to identify KEGG pathways that are statistically enriched in mutated genes of identified pathways compared with the human genome background, and to assess correlations between pathway groups and therapy.

Results

Collaborative pathway groups from hormone therapy and chemotherapy patients are mutually exclusive

Altered physiologic functions in CT and HT groups were examined separately using MUDPAC. We identified three collaborative pathways (Fig. 2A) in CT, having an MCR of approximately 79%, which means these three pathways are altered in the same 44 (of 56) chemotherapy patients. Of the 51 patients who received only hormone therapy, 20 collaborative pathways (Fig. 2B) were identified with an MCR of 57%, which results in a much longer and more stable plateau. None of these 20 are among the three pathways that form the CT group (Fig. 2C).

All three mutated pathways (PI3K-Akt signaling, p53 signaling, Wnt signaling) in the chemotherapy pathway group (CTPG) are TP53 related. This is consistent with the sample distribution within this therapy group, which has 34 (61%)
basal-like samples dominated by the mutated TP53 suppressor gene (21), which is known to be associated with the basal-like subtype.

In contrast with these three pathways, which play key roles in signal transduction and cell growth (Fig. 2D), the 20 pathways in the hormone therapy pathway group (HTPG) tend to have an organismal systems classification (Fig. 2D), including the endocrine, immune, central nervous, development, and digestive systems. More than half are either immune system or endocrine system related: 30% for the former (six pathways: Fc gamma R-mediated phagocytosis, chemokine signaling, B-cell receptor signaling, Toll-like receptor signaling, T-cell receptor signaling, leukocyte transendothelial migration) and 20% for the latter (four pathways: insulin signaling, estrogen signaling, progesterone-mediated oocyte maturation, prolactin signaling).

The gene sets that trigger pathway dysfunction in the two therapy groups are largely distinct from one another. The top 10 genes with highest mutation frequency in CT and HT are shown in Table 1.

Of the 10 mutated genes in each group that occur with the highest frequency, 3—TP53, PIK3CA, OBSCN—are common to the two groups. TP53 ranks first with a frequency of 73% in the CT but 18% (frequency of 8%) in HT. PIK3CA ranks first in HT with a frequency of 53%, but has a frequency of only 18% in CT. OBSCN has comparable mutation rates in the two groups. However, the mutation type and functional mutational score for OBSCN are different in the two groups. OBSCN has 10 mutations (one silent, six missense mutations, one frame shift mutation, two nonstop mutations) in CT with an average mutation score of 1.84, but it only has five mutations (two silent mutations, two missense mutations, one nonsense mutation) in HT with an average functional score of 0.44. This suggests that comparable frequencies do not necessarily imply comparable physiologic impact.

We also compared all the genes in the identified pathways of the two therapy groups with 87 plausible breast cancer genes (25): 37 confirmed drivers, and another 50 driver candidates identified with high likelihood computationally. Of the 532 genes in the CTPG, 27 are among the 87 plausible drivers (Fisher exact test \( P < 10^{-15} \)). Of the 1,059 genes in the HTPG, 40 are among the 87 plausible drivers (Fisher exact test \( P < 10^{-15} \)). These results suggest that the mutated genes in the identified pathways are highly enriched in drivers. For the two sets of drivers—27 genes for CTPG and 40 for HTPG—21 are common. The remaining 6 (19) are unique to the chemo (hormone) therapy patients.

### Table 1. Top 10 highly mutated genes in collaborative pathways of chemotherapy and hormone therapy groups

<table>
<thead>
<tr>
<th>Gene rank</th>
<th>Chemotherapy</th>
<th>Hormone therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TP53 (75%)</td>
<td>PIK3CA (53%)</td>
</tr>
<tr>
<td>2</td>
<td>TTN (21%)</td>
<td>MAP2K1 (20%)</td>
</tr>
<tr>
<td>3</td>
<td>PIK3CA (18%)</td>
<td>GATA3 (10%)</td>
</tr>
<tr>
<td>4</td>
<td>OBSCN (13%)</td>
<td>MLH1 (10%)</td>
</tr>
<tr>
<td>5</td>
<td>MUC4 (11%)</td>
<td>OBSCN (10%)</td>
</tr>
<tr>
<td>6</td>
<td>USH2A (11%)</td>
<td>LAMAS (8%)</td>
</tr>
<tr>
<td>7</td>
<td>AHCTRT1 (9%)</td>
<td>PHK2 (8%)</td>
</tr>
<tr>
<td>8</td>
<td>CACNA1B (9%)</td>
<td>RYR2 (8%)</td>
</tr>
<tr>
<td>9</td>
<td>CSMD2 (9%)</td>
<td>SPEN (8%)</td>
</tr>
<tr>
<td>10</td>
<td>DNAHS (9%)</td>
<td>TP53 (8%)</td>
</tr>
</tbody>
</table>

NOTE: Genes are listed with mutation frequency descending order, with mutation frequency shown in brackets.

Figure 2.
Mutated pathways in chemotherapy and hormone therapy. A, mutated pathway collaboration in chemotherapy: 79% of chemo patients can have three pathways simultaneously mutated. B, mutated pathway collaboration in hormone therapy: 57% of hormone patients can have 20 pathways simultaneously mutated. C, Venn diagram of two pathway sets showing there is no common pathway between different therapy treatments. D, relationships between identified pathways and their KEGG pathway category, with each column is the identified pathway ID and each row represents a KEGG pathway category with higher level category bracketed on right. A red cell means this pathway belongs to the certain category, otherwise not.
Pathway groups are correlates of therapy recommendations

The choice of adjuvant therapy, as noted above, generally follows subtype (4): the Luminal-A subtype is treated with hormone therapy; the Luminal-B subtype with chemotherapy, but occasionally with hormone therapy, and the HER2 and basal-like subtypes with chemotherapy.

The patient population in this analysis deviates somewhat from these guidelines, showing 9 of the 49 luminal-A patients are in chemotherapy. The deviation for luminal-B is more severe, with only 3 of the 13 in chemotherapy. We will comment on this below.

The subtypes and their therapy recommendations are approximately correlated. Thus, for basal-like subtype patients having chemotherapy, 95 of 102 pathways (34 basal-like patients × 3 identified pathways in chemotherapy) are mutated in CT while 7 are not; 264 out of 680 (34 basal-like patients × 20 identified pathways in hormone therapy) pathways are mutated in HT while 416 are not. Consequently, basal-like patients having chemotherapy are significantly more likely to be mutated in CTPG than in HTPG (P < 10−15). Similar results are observed for HER2+ (P = 7.35e−4) and Luminal-A (P = 2.10e−8), which are recommended for chemotherapy and hormone therapy, respectively. But there are no significant correlations found in Luminal-B patients, no matter in CT (P = 0.6119) or in HT (P = 0.7489).

There are, as expected, strong correlations between the mutated pathway groups seen in patients and the kind of therapy they receive. The distribution of mutations between HTPG and CTPG for CT patients is highly significant. In particular, the probability that the observed distribution of mutations between the two pathway groups due to chance, given the patients are in chemotherapy (Fig. 3A), is P < 10−15. This follows from a Fisher exact test, based on the observation that for CT (i) 141 of 168 (56 × 3) CTPG pathways are mutated and 27 are not; and (ii) 503 of 1,120 (56 × 20) HTPG pathways are mutated and 617 are not (Fig. 3A; CT columns).

Similar results apply to patients receiving hormone therapy (Fig. 3A; HT columns): 69 of 153 (51 × 3) CTPG pathways are mutated while 84 are not, and 677 of 1,020 (51 × 20) HTPG pathways are mutated while 343 of them not. The probability that mutations are equally likely in the two pathway groups, given that patients are in hormone therapy, is P = 4 × 10−7.

These results provide a previously unmodeled molecular rationale for the choice of therapy based on cancer subtype. In addition, as discussed below, the structure of Fig. 3A suggests two distinct groups: 29 patients have almost all 20 pathways mutated while 84 are not, and 677 of 1,020 (51 × 20) HTPG pathways are mutated while 343 of them not. The probability that mutations are equally likely in the two pathway groups, given that patients are in hormone therapy, is P = 4 × 10−7.

Pathway groups stratify subtypes

The Luminal-A pattern provides strong statistical evidence that the population of Luminal-A patients in this study can be split into two distinct groups: 29 patients have almost all 20 pathways from HTPG mutated (577 mutated, 3 not), whereas 17 patients show relatively infrequent mutations (65 mutated, 275 not). The probability that this pattern is the result of chance is P < 10−15. This again suggests that traditional subtypes can be stratified further and that higher resolution can be identified by pathway groups. Similarly, although the HER2+ pattern does not support correlation with traditional clinical guidelines for therapy, it does suggest two distinct subpopulations.

Discussion

We demonstrated that HT and CT samples are characterized by mutually exclusive sets of mutated pathways that are strong correlates of subtype-based therapy recommendation. Furthermore, genes in these altered pathways are highly enriched in breast cancer drivers and may provide further guidelines for the personalized therapy. Because all the mutations reported in this article are from DNA sequenced and annotated prior to surgery or therapy, using tissue from the primary tumor at the initial site of the cancer, the results are not a consequence of mutations that might be induced by therapy.

The discovery that the altered cellular processes that drive transformation in patients having hormone therapy have a substantially different biology than those that do not may open an opportunity for the identification of new therapies. Particularly, we found that 100% of the pathways in chemotherapy are TP53 related, whereas 70% of pathways in hormone therapy are involved in organismal system, especially in immune system (30%) and endocrine system (20%).

Both immune (26, 27) and endocrine systems (28–30) can work primarily through ERs, regulating cells in pathways from these two systems could therefore lead to the successful

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development of therapeutic concepts in breast cancer. In brief, we hope our identified pathways could be considered as a signature to classify patients for their treatments in the future, as an auxiliary of current protocol, after we could get enough samples to test. Moreover, speaking of each individual therapy option, like hormone therapy for example, beyond targeting estrogen-related genes to lower or stop estrogen level, our mutated pathways also evidenced by our identified pathways tend to be great help physicians to draw up clinical schemes, which are greatly helped physicians to draw up clinical schemes, which are greatly helped physicians to draw up clinical schemes, which are greatly helped physicians to draw up clinical schemes, which are greatly helped physicians to draw up clinical schemes, which are greatly helped physicians to draw up clinical schemes, which are.

The mutated genes in our identified pathways tend to be sample specific (Fig. 3B). Except for TP53 and PIK3CA, which have the highest mutated rates in chemotherapy (75% in CT and 8% in HT) and hormone therapy (53% in HT and 18% in CT) groups, respectively, almost all the other genes are dispersed among samples, even for subtypes that have similar pathway patterns. But the mutated genes in these pathways are specific to each patient: there are totally 12 genes mutated in HER2+ (PIK3CA, TP53, ABL2, ATR, NKP2, TXK, XCR1, ADCY8, FLT4, PPP3CA, STAT5B, IRF9), total 15 genes mutated in Luminal-B (PIK3CA, TP53, RPS6KA6, TCF7, FGF, PAK7, PIK3R1, BCL2L11, CCNB1, FGFR2, FN1, MYH10, NFATC4, PIK3R3, TRIP10). Besides PIK3CA, which is mutated in all 7 patients, and TP53, which is mutated in 6 of 7 patients, all the remaining genes are mutated in only 1 patient, and no two genes are mutated in the same patient.

The conventional way to determine whether to apply chemotherapy or hormone therapy is according to the breast cancer molecular subtypes, which are induced by gene regulation level of several molecular factors like ER, PR, and human epidermal growth factor. This strategy has been developed for decades and greatly helped physicians to draw up clinical schemes, which are also evidenced by our identified mutated pathways: different molecular subtypes tend to have distinct mutation patterns and are approximately concordant with pathways of their recommended therapy groups.
The subtype information, including biologic process and molecular functions in epigenetic alone, however, may not fully uncover the whole picture. Mutated drivers, including both genes and pathways that initiate cancer development, may supplement this picture. We present in Results that even for the same subtype like Luminal-A, the mutational landscape is diverse between patients in CT and HT. This may advise that current treatment protocol need to be personalized by integrating genetic factors like driver genes and driver pathways.

One limitation of this article is what we reported are exploratory results only. We discussed summarized clinical information from TCGA with observation and detailed interpretation, but without very strong statistical support due to small portion of available samples in TCGA (around 50 samples for each therapy), and without further verification in new samples due to limited existing clinical sources.

Taken together, our findings suggest that systematic mutation analysis of breast cancer can reveal pathway dysfunction status and mutated gene portraits that may not easily be discovered in transcriptome level. This brings new insights about personalized treatment and targeted agents.

**References**


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