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Original article

What to expect from high throughput genomics in metastatic breast cancers?

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ABSTRACT

Breast cancer is a heterogeneous disease and its genomic characteristics have been widely studied in the last years. Although several progresses have been made, metastatic disease is still incurable in the majority of patients. Recent genomic studies have shown that a large number of candidate targets exist in breast cancer. Currently only two drivers have been validated (ER and HER2), but several others seem to be associated with objective response, such as PIK3CA mutations, FGFR1 amplifications, AKT1 mutations, EGFR amplifications and ERBB2 mutations. Beside driver identification, many other applications can be developed for genomics such as identification of lethal subclones, DNA repair defects or immune response against tumor. Most of the precision medicine programs currently use targeted sequencing. Nevertheless, whole exome sequencing, RNA sequencing, gene expression analysis, phosphoprotein detection, SNP arrays and ctDNA sequencing have been also proposed in clinical trials.

Introduction

Numerous models to explain cancer complexity have been proposed up to now, such as that based on the principle of progressive accumulation of DNA mutations, responsible for initiation, progression, dissemination, response and resistance to treatment [1,2]. Somatic mutations are abundant in every cancer cells, but most of them do not play a role in cancer progression. They are classified in “passengers”, that have not a functional effect but are associated with a clonal expansion; “drivers”, associated with a selective advantage; “actionables”, that have diagnostic, prognostic or therapeutic significance; and “druggables”, that are targets of therapeutic development [3,4]. In this context, multigene diagnostic assays, such as next generation sequencing (NGS) technologies or comparative genomic hybridization arrays (CGH), assume primary importance in cancer treatment development [5–7]. Thus genomic technologies are relevant and their applications are growing. Targeting oncogenic drivers can be a possible approach to improve outcome in patient's refractory to standard treatments. Therefore, new clinical trials are required to translate scientific innovations to clinical practice for patients selected according to their molecular characteristics [8].

Breast cancer is the leading cause of cancer related death worldwide, with about 232.340 new cases of invasive breast cancers and 390.620 deaths in the USA in 2013 [9]. Although in the last years advances have been made in breast cancer management, metastatic disease is still incurable. The majority of progress has been done for targeted therapies, for instance, therapies targeting HER2 with pertuzumab or trastuzumab-emtansine [10,11]. However, studies on molecular characterization have shown that breast cancers present many other genomic alterations, including AKT1, PIK3CA and FGFR1, which can be targeted by new molecular drugs [6,12,13].

In this paper we will analyze different applications of high throughput genomic assays in metastatic breast cancers and their involvement in therapeutic development of several targeted agents.

Genomic tests to decipher cancer biology at the individual level

Oncogenic drivers

Identification of drivers at the DNA level.

In the last years the concept of precision medicine has been growing. The introduction of high throughput technologies, such as

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whole exome sequencing and copy number analyses has allowed to identify tumor molecular characteristics. The introduction of these techniques advantaged the discovery and update of oncogenic drivers. Targeting drivers gene mutations can lead to oncogene de-addiction and tumor shrinkage. The SAFIR-01 trial has been conducted in this context: guide patients towards specific targeted therapies according to their genetic characteristics [6].

Over the past few years, many other trials have emerged to study genomic characteristics in breast cancer patients, with the identification of about 10–20 candidate actionable drivers, most of them occurring in less than 10% of patients [12–16]. Many clinical trials have been focused on these new targets, but nowadays only few of them are associated with objective response in clinical studies. Among these we can mention PIK3CA mutations, observed most commonly in hormone receptor positive breast cancers (34,5%) respect to HER2 positive (22,7%) or basal-like tumor (8,3%) [17]. In this setting of patients, specific treatment with BYL719, a selective PI3Kα inhibitor, in combination with endocrine therapy (letrozole or exemestane) has shown promising results in a phase I study, associated with manageable adverse events [18]. FGFR1 amplifications, present in about 10% of breast cancer patients and associated with a poor prognosis, showed encouraging results in preclinical studies. There are nowadays several ongoing clinical trials with different compounds, such as lucitanib, dovitinib, AZD4547, nintedanib, BGJ398 and JNJ-42756493 [19–21]. Other rarer gene alterations in breast cancer, such as AKT1 and ERBB2 mutation and EGFR amplification, are present. Mutations of AKT1 occur in about 4% of breast cancers. Targeting mTOR pathway with specific inhibitors, such as everolimus, leads to promising results in clinical trials [6,22]. ERBB2 mutation is an uncommon alternative mechanism to activate HER2 in breast cancer and is currently considered as a possible target of the HER2 inhibitor neratinib [23]. Finally, EGFR amplification, observed in 2% of the cases, can be the target of specific EGFR inhibitors [6]. Unfortunately, other identified gene alterations have not shown encouraging results in clinical studies. For example, the amplification of CCND1 is not associated with a better response in patients treated with palbociclib, a cyclin-dependent kinases 4 and 6 inhibitor, compared to unselected patients. Nevertheless this targeted therapy can improve progression free survival in advanced breast cancer, independently of the CCND1 abnormality, explaining why a phase III trial is ongoing (NCT01942135, www.clinicaltrials.gov) [24]. The presence of co-existing mutations in the same tumor can be associated with resistance to therapy. Hortobagyi and colleagues observed a better response in patients with a mutation activating mTOR pathway, such as PIK3CA, FGFR1 and CCND1, compared to patients not carrying gene mutations. Also a reduction of everolimus efficacy in patients carrying multiple mutations was detected [25].

In summary, in advanced breast cancers, five gene alterations have been associated with objective response: PI3KCA mutations, FGFR1 amplifications, AKT1 mutations, EGFR amplifications and ERBB2 mutations. Five to ten other genes are candidate drivers based on preclinical studies.

Currently, the preferred approach for personalized medicine is to use targeted sequencing and copy number analyses in order to deliver the best treatment.

Targeting the pathways: identification of driver at the RNA/protein level

While most of the current strategies of personalized medicine aim to identify DNA alterations, other approaches could identify pathway activation and dependency. PI3K/mTOR pathway activation is involved in resistance to hormonotherapy in ER-positive breast cancer. The BOLERO-2 trial results highlight that targeting mTOR pathway is an effective strategy to overcome resistance to a previous endocrine therapy [26]. Loi and colleagues identified that mTOR activation was associated with better response with mTOR inhibitor treatment [27,28]. Gene expression could be useful to identify drivers of cancer progression and pathway dependency.

Another pathway driving cancer progression is the CDK4/RB pathway, associated with cell cycle progression, through transcription activation of E2F-regulated genes. Abnormal RB pathway is reported to be aberrant in about 20–35% of breast cancers and is associated with poor outcome. It responds better to cytotoxic drugs than to tamoxifen, due to the bypass of antiestrogen signaling. Therefore, RB pathway can be studied as a biomarker to address the therapeutic choice in ER-positive breast cancers [29].

ER, mTOR and CDK4 pathways drive cancer progression in a majority of ER+/Her2- BC. Thus, there is a need to develop molecular tools to assess pathway activation and dependency by gene expression arrays and phosphoprotein arrays.

Lethal subclones and intratumor heterogeneity

Profiling tumors emphasizes that genetic differences exist not only between different tumors, but also in the same tumor. This intra-tumor heterogeneity is related to genomic instability, causing an additional challenge for developing drugs in presence of different mutations [30,31]. Within the tumor multiple differences at genetic and epigenetic levels exist, resulting in therapy resistance. Indeed, Landau and colleagues worked on chronic lymphocytic leukemias and have shown different responses to treatment based on DNA methylation tumor profile at an epigenetic level [32]. However genomic instability results in dynamic alterations in clonal and subclonal frequencies. Selective pressure, determined by treatment and tumor microenvironment, leads to a selection of specific subclones responsible of resistance to target therapy [33]. Lethal subclones can be present in a minority of cells in primary tumor. Ultradeep sequencing could allow early identification and provide the information for specific treatment [34]. Several studies have shown that targets can be lost or gained during tumor evolution, putting in evidence the need of a new biopsy at the time of disease progression [35]. Currently the re-biopsy is not a clinical practice due to the invasiveness of the procedure. Recent studies have shown that genomic changes occurring during the course of the disease can be detected with liquid biopsy, by sequencing circulating DNA at baseline and at disease progression. Circulating DNA could monitor the appearance of lethal clones, without invasive procedures [36].

Several gene alterations such as ESR1, TSC1/2 and PTEN have been associated with resistance genotype. To illustrate, Arnedos et al. observed that metastatic breast cancers in comparison with the primary tumors present higher level of genes involved in migration processes or in resistance mechanisms, such as ESR1 or TSC1 [37]. Toy and colleagues observed that the ESR1 was frequently mutated at metastatic site, but not in primary tumor samples. Overall the mutation was detected in less than 1% of early breast cancer and in about 20% of aromatase inhibitor resistant tumors and is associated with a poor prognosis. They identified that the mutation involves the ligand binding domain of ESR1, which is responsible of acquired resistance to endocrine therapy through the activation independent to the ligand. However, drugs directly targeting ER and inducing its proteasomal degradation, such as fulvestrant, may be effective against the mutant forms of estrogen receptor [38,39]. Moreover, PTEN mutations and deletions could be a mechanism of resistance to PI3K inhibitors. Indeed, the progressive decrease or loss of PTEN during treatment with BYL719 might lead to PI3K/AKT pathway activation and consequently to tumor growth [40].

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