Heterogeneity in Breast cancer

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Abstract

Breast cancer is malignant tumor occurred on breast epithelial tissue, although the mortality rate of breast cancer decreases, it remains one of the highest mortality rate diseases. Ignoring few exceptions, almost all tumors are derived from one single cancer cell. However, in clinical diagnosis, most human tumors demonstrate amazing heterogeneity in many morphological and physical characteristics. At the molecular level, human breast cancer is a complex heterogeneous disease in natural history. The combination of molecular origin and malignant cells and the diversity of hosting environment created the tumor subpopulations, which have different phenotypic characteristics. These subpopulations have different responses to the treatment and clinical outcomes. According to the combined affection of genetic and epigenetic instability and therapeutic interventions, different evolutionary routes will be generated thereby caused the inevitable tumor heterogeneity. In this review, the heterogeneous indication of genetic and epigenetic and metastatic as well as the potential causes and their effect for the clinical management of breast cancer were discussed.

1 Breast cancer heterogeneity

Among many breast cancer typing methods, China most frequently use histological types including non-invasive cancer, early invasive cancer, invasive special cancer, invasive non-special cancer and other rare cancer. Here, invasive non-special cancer, which has a low level of differentiation and poor prognosis in comparison to other cancers, is the most common type accounted for 80% in human breast cancer. However, judging prognosis still need integrate the stage of disease and other factors. Surprisingly, the phenomenon that the prognosis of the breast cancer patients in the same histological type and the same clinical stage has a big difference even after suffering the same treatment demonstrated breast cancer was a highly heterogeneous malignancy. Along with progress in molecular biology, medical research began to enter molecular era as the traditional histopathological classification couldn’t fulfill current tumor researches and treatment needs, and meanwhile breast cancer molecular typing which based on genetic profiles and molecular biological characteristics provided a necessary supplement for breast cancer classification. In 2000, Perou et al. identified four groups of mammary epithelial samples, which containing ER positive/luminal-like, human epidermal growth factor (HER2) positive, basel-like and normal breast, based on pervasive differences in gene expression patterns(Perou et al., 2000). In 2011, experts at St.Gallen International Breast Cancer Conference reached a consensus that instead of gene microarray breast cancer was classified according to the ER, PR, HER2, Ki-67 results detection by immunohistochemical method into four categories: luminal A, luminal B, HER2 positive and triple-negative breast cancer (TNBC)(Goldhirsch et al., 2011). Even though breast cancer has entered the stage of molecular typing, there is a huge heterogeneity no matter prognosis or the response to the same treatment regimen in the same molecular subtype.

1.1 Genetic heterogeneity

Variations occurred in BRCA1 and BRCA2 have demonstrated that breast cancer phenotype was wrapped up in scattered mutation model in different populations. Exon sequencing for BRCA2 revealed
the emergence of five sequence variations, among them the four appearing on exon 11 were somatic and the other one which located on the UTR of exon 2 was germline mutations (Ayub et al., 2014). Laraqui et al. performed BRCA1 mutation analysis on 121 women with breast cancer in Morocco, and the result showed that only 31.6% of the patients with family history and 1% of early onset sporadic patients were related with BRCA1 mutation. The pathogenic mutations contains two frameshift mutations (c.798_799delITT, c.1016dupA), one missense mutation (c.5095C>T) and one nonsense mutation (c.4942A>T)(Laraqui et al., 2013). These findings reflected the genetic heterogeneity in Moroccan population. Cao W et al. screened BRCA1 germline mutation on 62 patients with familial breast cancer, and then checked out five deleterious mutations of which the mutation rate was 11.3% (7/62). They found two fresh mutations (3414delC and 5,280 C > T), two recurrent mutations (5,273 G > A and 5589del8)(Cao et al., 2013). Melchor L et al. analyzed DNA from 74 family patients and 19 sporadic breast cancer patients by array Comparative Genomic Hybridization (aCGH). Results suggested that BRCA1/2 sensitive tumors showed higher genomic instability compared to BRCA1/2 tumors and sporadic tumors, and that estrogen receptor(ER) negative tumors exhibited higher genomic instability and more differential variation regions in compared to ER positive tumors(Melchor et al., 2007).

1.2 Epigenetic heterogeneity
It should also be noted that, in addition to genetic variation, epigenetic events are heritable and could be suffered selection. Similar to genetic instability, epigenetic instability may be caused by the loss of function which can maintain the epigenome completeness. Epigenetic instability contributes to breast cancer phenotypes intratumoral heterogeneity. Chromatin structure and dynamic, which were influenced by epigenetic marks such as histone modifications and DNA methylation, play a decisive role in mediating gene expression. Hong CP et al. found that regulatory active elements identified by formaldehyde-assisted isolation of regulatory elements (FAIRE) method were highly correlated with histone modifications, for instance H3K4me3 and H3K9/16ac, by comparing the pattern of histone modifications with regulatory elements (Hong et al., 2012). Heyn et al. analyzed high resolution DNA methylation profiles in 15 pairs of twins with inconsistent breast cancer, and then identified 403 differential methylation CG sites in known and new potential breast cancer genes(Heyn et al., 2013). Jing Wei et al. analyzed the expression level of estrogen receptor alpha (ERα) and methylation status of four promoter regions of ERα in 113 familial breast cancer patients(Wei et al., 2012). ERα methylation only in 47(41.6%) patients could be observed, and the expression level of ERα was significantly associated with the ERα methylation in promoter regions. Besides that, ERα methylation was significantly associated with tumor size, PR expression, P53 nuclear reactors, BRCA1 and BRCA2 states. In brief, epigenetic changes of ERα may be implicated in the pathogenesis of familial breast cancer.

1.3 Metastasis heterogeneity
As one of the most frequently diagnosed cancers, breast cancer is the leading cause of cancer death in women worldwide. However, in these patients, the main cause of death is not primary tumor but distal metastases. As a complex multi-step process, cancer metastasis was driven, promoted, regulated by abnormality cellular signal. Masses of signaling pathways, such as Myc, β-catenin and TGF-β pathway, were determined to play critical roles in metastatic process of breast cancer. Despite progress, 20% to 30% of patients with early breast cancer would go through recurrence and distant metastases(Early Breast Cancer Trialists' Collaborative, 2005). Recurrent risk was affected by the stage of initial symptoms stage and the fundamental tumor biology. There were many independent risk factors of breast cancer recurrence, containing tumor size, node involvement, grade, lymphatic vascular invasion, the status of ER and HER2 and so on(Kennecke et al., 2010). As inability to accurately predict the risk of metastasis in individuals, 80% of the patients received adjuvant chemotherapy even though only about 40% of the patients would undergo relapse and then die of cancer metastases(Weigelt et al., 2005). Thus, many patients that could be cured by local treatment such as surgery therapy and radiation therapy conducted over treatment resulting in suffering unnecessary side effects induced by chemotherapy.
Many studies believed that metastatic ability was the final step in tumor progression (Hanahan and Weinberg, 2000), and implied that genes in metastatic tumor cells should be similar to the genes in primary tumor cells. Although there was a close genetic relationship between cells in primary tumor and metastatic tumor, different mutations appeared on breast cancer primary and metastatic tumor, suggesting the genetic diversity between them (Kuukasjarvi et al., 1997; Torres et al., 2007). Different subtypes of breast cancer also have different metastatic behaviors. Research found that there was a difference in metastatic time, the cumulative incidence of brain metastases at 2 and 5 years were 5.6% and 9.6% in 679 nonmetastatic TNBC patients, respectively (Dawood et al., 2009).

For purpose of the incidence of brain metastases in HER2 overexpression patients, Gabos Z et al. analyzed 301 HER2 positive and 363 HER2 negative patients. The incidence of brain metastases in HER2 overexpression patients was 9% while in HER2 negative was only 1.9% (Gabos et al., 2006). Hagen K et al. found triple negative nonbasal tumor groups exhibited brain metastases rate of 7.2% (Kennecke et al., 2010), whereas basal like tumors exhibited different rates of 10.9%, 18.5%, 16.6%, 17.2% and 9.3%, respectively, in brain, lung, bone, distant nodal and liver metastases.

2 The reason for breast cancer heterogeneity

With one or two exceptions, spontaneous tumors are differentiation from a unique tumor cell (Marusyk and Polyak, 2010). However, when clinical diagnosis, most human tumors exhibit amazing heterogeneity in many morphological and physical characteristics, such as the expression of cell surface receptor, proliferation and the potential of angiogenesis. To a large extent, the heterogeneity may be attributed to morphological and epigenetic plasticity. Moreover, strong evidence suggested that genetic differential tumor cell clones coexisted in tumor tissues. Clonal selection theory was one of the widespread agreement tumor heterogeneity formation mechanisms. Tumor cell was originated from single clone, and the clone with survival or growth advantage gradually became the main portion of tumor cell population through natural selection mechanism (Nowell, 1976). In the process of tumor formation, tumor cell would experience generations of division and reproduction with gene mutation or other biological macromolecules changes, which further shaped a great deal of differences and diversities in growth rate, invasive ability, responding capability of growth signals and chemical drug susceptibility. Tumor heterogeneity was the result and external manifestation for clonal selection and somatic mutation while genetic and epigenetic changes in tumor cell, which determined the somatic mutation and natural selection rate, was the internal basis for tumor heterogeneity.

Tumorigenesis is an evolutionary process, which driven by Darwinian selection and accelerated by novel mutations (Michor et al., 2004). The results of human cancer genome sequencing indicated that there was a high genetic heterogeneity between tumor cells. Extensive somatic mutations took place in the tumorigenesis. For another, the disorder of DNA damage repair function, which could maintain genomic stability under normal circumstances, would accelerate mutation and random choices opportunity leading to tumor heterogeneity (Bartkova et al., 2005). In most cases, tumor growth is believed to be driven by the most advanced cancer cell subpopulation which carried the largest number of cancer driven mutations. However, that many variations presenting a low frequency indicated tumor involved a plurality of subclones. Currently, the relevance of these subclones could not be fully understood.

Increasingly studies show that there is a subset of tumor cell subpopulations that can self-renew, induce cancer cell differentiation, proliferation, metastasis and relapse, resist to chemotherapy in breast cancer cells, called cancer stem cells (Pietras, 2011; Campbell and Polyak, 2007). In other words, actually tumors are composed of cancer stem cells with infinite self-renewal capacity and the cell mass with the unbalanced differentiation produced by cancer stem cells. This model has long been thought to be the important mechanism causing the phenotypic and functional heterogeneity and tumor diversity, simultaneously has been considered to be the sole source of tumor recurrence. Recent studies have found that there is an immense difference in the phenotype of tumor stem cells among different individuals even with the same tumor type, meanwhile there are multiple tumor stem cells with significant differential phenotypes or genotypes. In the meantime, that breast cancer tissue involved heterogeneous breast cancer...
stem cells may be the main cause of breast cancer heterogeneity.

3 The impact of breast cancer heterogeneity for clinical treatment

The conduction of early diagnosis and comprehensive treatment can significantly reduce breast cancer mortality rate. On account of breast cancer heterogeneity, patients with the same histological type and similar differentiation may have different biological characteristics and prognosis, patients with consistent pathological staging have different prognosis, and even the patients with the uniform molecular type vary in reaction to the identical treatment options. As a highly heterogeneous tumor, different breast cancer patients should accept individualized treatment in accordance with their different genetic and epigenetic features in order to solve the current predicament of breast cancer treatment.

Benz et al. found breast cancer cell MCF7 accompanied with HER2 positive, which had a dependence on estrogen yet a drug resistance to tamoxifen (TAM), even could accelerate the transfer rate of certain cancer cells(Benz et al., 1992). TAM had an efficiency of 48% for the patients with ER positive, HER2 negative, 20% for the patients with ER positive, HER2 positive, whereas had no therapeutic significance for the patients with ER positive, HER2 positive, PR positive. About 20% to 30% of breast cancer patients were HER2 overexpression, and monoclonal antibody trastuzumab against HER2 overexpression was on the market in 1998. The monoclonal antibody Herceptin specific to HER2 could reduce the risk of relapse of breast cancer positive for HER2, significantly(Chang, 2010). However, endocrine therapy and trastuzumab targeted therapy for TNBC were ineffective. Platinum drugs could cause DNA double strand breaks (DSBs), impede DNA replication, transcription, and then result in cell death ultimately. BRCA1 was related to the repair of DSBs, therefore platinum drugs may be effective in TNBC with BRAC1 mutation(Vollebergh et al., 2011). Currently, that breast cancer treatment developed from single surgery to multidisciplinary treatment significantly improved the prognosis of breast cancer.

Breast cancer stem cells have a resistance to traditional clinical treatment means and always lead to recurrence and metastasis resulting in the failure of treatment. Researchers found that radiation therapy not only could kill half of the tumor cells but also could transform other tumor cells to cancer stem cells resistant to treatment(Lagadec et al., 2012). Conley SJ et al. found that potential breast cancer drugs Avastin and Sutent, which delayed the rate of tumor worsened by blocking blood vessel growth, could increase the number of cancer stem cells in mouse mammary tumor causing the growth and diffusion of breast cancer once again(Conley et al., 2012). Thereby, if the treatment of breast cancer stem cells was ignored, tumor recurrence and metastasis would be more likely to occur. Cancer stem cells phenotype had significant variations in different breast cancer patients, and a patient could carry several breast cancer stem cells.

Cancer is highly heterogeneous, if only contrapose to portion of breast cancer cells or individual genetic features when treat breast cancer, breast cancer could not be cured entirely. Currently, combination therapy involved genetic, epigenetic and cancer stem cells may be the best treatment method. Whatever, this review provided a new research direction for breast cancer and treatment.

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