Trends in Cancer Research
Vol. 9, 2013

Mini-Review

Metaplastic breast cancer - a rare but challenging entity

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ABSTRACT

Metaplastic breast carcinoma is a rare type of breast cancer. Due largely to the lack of drug targets and the high heterogeneity of tumor components, metaplastic breast cancers are difficult to manage. The incidence of metaplastic breast cancer has been rising during the past decade, becoming the leading cause of cancer-related death among women in the Western world. However, there is no standard treatment for this subgroup of breast cancer. Individualized genetic profiling may provide promising targets for therapeutic development. The transcriptional regulator p63, a member of the p53 tumor suppressor family, appears to play a key role in the maintenance and regeneration of epithelial stem cells and is actively involved in the pathogenesis of metaplastic breast carcinoma. Positivity of p63 has been applied in the diagnosis of metaplastic carcinomas of the breast. Further explorations are necessary to reveal the mechanisms underlying p63 upregulation in metaplastic breast carcinomas and the downstream gene targets of this protein, which will facilitate the therapeutic development for this disease.

KEYWORDS: metaplastic breast cancer, basal-like, p63, Wnt/β-catenin

INTRODUCTION

Breast carcinoma is the most frequently diagnosed cancer and leading cause of cancer death in women. Each year, about 1.4 million new cases of breast carcinoma are diagnosed worldwide and over 450,000 women die of this disease [1]. According to the American Cancer Society, deaths due to breast carcinoma in the age groups of 20 to 39, 40 to 59, 60 to 79 and ≥80 range from 2.69%, 28.65%, 41.63% and 27.03%, respectively [2]. Metastatic disease, or the spread of tumor cells throughout the body, is the main cause of mammary tumor-related deaths.

Breast epithelium is composed of an inner luminal layer and a surrounding basal layer. Gene-expression profiling distinguishes the molecular subclasses of breast cancers as the luminal A and B, erbB-2 overexpressing, normal breast-like and basal epithelial-like tumors, which show significantly different outcomes in patients [3]. Basal-like breast cancers are associated with worse overall and disease-free survival compared with other subtypes. In addition, there is an immunohistochemical surrogate for the basal-like profile, including markedly reduced expression of estrogen receptor (ER) and erbB-2, but positive staining of proteins that are characteristic of the normal myoepithelial cells, such as epidermal growth factor receptor (EGFR) and basal cytokeratins (CK) 5/6 [4]. Due to the lack of molecular targets commonly used in targeted therapy, this group of tumors are more difficult to treat.

Metaplastic carcinoma, a subset of basal-like breast cancers, is characterized by the histologic presence of two or more cellular types, rapid growth and large size, as well as high grade and poor prognosis [5, 6]. It has a high hematogenous metastatic potential to lung and bone, as well as liver and brain, rather than lymphatic spread [7-9]. In the current World Health Organization classification, metaplastic carcinoma includes squamous cell carcinoma,

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adenocarcinoma with spindle cell differentiation, adenosquamous carcinoma, mucoepidermoid carcinoma, and carcinoma with mesenchymal elements (carcinoma with chondroid or osseous metaplasia and carcinosarcoma). Metaplastic carcinoma often presents in women above 50 years of age [10]. It is a rare form (less than 1% diagnosed annually) of breast cancer relative to invasive ductal carcinoma (IDC). Patients with metaplastic carcinoma have been treated using similar regimens as IDC, however, with worse outcomes [11]. The phenotypically diversified subclones in metaplastic carcinoma are resistant to conventional chemotherapy [12]. Hormonal therapy is as ineffective as chemotherapy, due to the negative expression of ER and Her-2/neu. Mastectomy is performed more often for patients with metaplastic breast carcinoma, as their tumors are usually large (>4 cm) at presentation. There are few therapeutic options once disease recurs or progresses. Thus, more courageous treatment strategies and therapeutic targets are required to improve survival rates.

This review focuses on the biological features and pathogenesis of metaplastic breast carcinoma, and discusses the potential novel therapeutic targets based on the genetic mechanisms and changes of biomarker expressions.

Metaplastic carcinoma, a distinct entity of basal-like mammary tumors

Metaplastic carcinomas were first described in 1973 as mammary tumors with mixed epithelial and sarcomatoid components [13]. They generally have basal-like immunophenotype, including the negativity for ER (82-100%) and rare overexpression of HER2 (a positive rate of 7-14%) [14]. Progesterone receptor is present in ~21% of patients with metaplastic breast carcinoma [6]. Although there is a marked parallelism between basal-like and triple negative breast cancers [15], metaplastic carcinomas show distinctive histomorphologic features and molecular signatures compared to the latter. The 5-year overall (54.5%) and disease-free (45.5%) survival rates in metaplastic breast carcinomas are significantly lower than those (73.3% and 60.3%, respectively) in triple-negative IDC [8]. In general, metaplastic carcinomas have a poorer prognosis than triple-negative IDC, despite adjuvant chemotherapy [16]. Moreover, non-triple negative metaplastic carcinomas have poorer prognoses and are more aggressive than triple negative metaplastic carcinomas [17].

In addition to ER and HER2 negativity, CK5, CK14 and EGFR are good markers for the identification of basal-like and metaplastic carcinomas [18]. A subset of expression markers that have myoepithelial origin including smooth muscle actin (SMA, 60%), p63 (57-86%), CD10 (85%), S100 (45%) and laminin 5 (96%) are specific for metaplastic carcinomas [19-21]. p63 is strongly expressed in metaplastic carcinomas and highly specific for those with spindle cell and/or squamous differentiation, whereas only 1 of 174 (0.6%) non-metaplastic invasive carcinomas show positive staining of this protein [22]. The sensitivity and specificity of p63 as a diagnostic marker for metaplastic carcinoma are 86.7% and 99.4%, respectively. p63 expression is present in all squamous carcinomas that are also CK5/6 positive and ER/PR/HER2 negative.

The mesenchymal components of metaplastic carcinomas are characterized by negative/low expression of claudin and E-cadherin, but high expression of vimentin [23, 24]. Approximately 70% of metaplastic carcinomas show *EGFR* gene amplification and overexpression in the squamous component, which may have treatment implications in targeted therapy [25-27]. The expressions of MUC1 and beta-catenin are often absent or aberrant, which favors the metastatic dissemination of metaplastic breast carcinomas [5]. The small heat shock protein alpha B crystallin is commonly expressed in basal-like tumors and contributes to the aggressive phenotype. A higher rate of alpha B crystallin expression is detected in metaplastic breast carcinomas (68% sensitivity and 88% specificity [28]), suggesting that these tumors represent a histologically distinctive subset of basal-like breast tumors [29].

Origins of metaplastic breast carcinomas

Although specific tumor subtypes can be distinguished by the histomorphologic features and molecular signatures, the cellular origin of metaplastic breast carcinomas has not been defined. There is no good concordance of outcome predictions for the cancer patients. Oberman reported that the size of the neoplasm rather than the microscopic patterns at the time of initial treatment

correlated with the prognosis in patients with metaplastic breast carcinomas, suggesting that they are variants of a single entity [30]. There are molecular studies supporting an origin from myoepithelial cells [20], a metaplastic (trans-differentiation) process involving the sarcomatous component converted from a carcinomatous component [31-33], or the neoplastic transformation from a totipotent stem cell [19]. Metaplastic carcinoma of the breast as a second neoplasm has also been reported [34].

Metaplastic transition could be traced from cells within the epithelial nests to those within the sarcomatous lobules. Ultrastructurally, cells in the former region showed epithelial characteristics and those in the latter region, mesenchymal and/or cartilaginous features. Myoepithelial cells constitute the basal cell layer of normal mammary epithelia. Myoepithelial carcinoma or carcinoma with myoepithelial differentiation exhibits a partial or total spindle growth pattern, or a distinctive form of "matrix-producing carcinoma" [32]. In metaplastic matrix-producing and spindle cell carcinomas, immunohistochemical evidence of myoepithelial differentiation has been demonstrated with at least two conventional myoepithelial markers being positive in every case [35]. Compared to benign myoepithelial tumors, p53 overexpression is seen frequently in metaplastic matrix-producing and spindle cell carcinomas [36]. The actomyosin positive cells with structural appearances of myoepithelial cells in a variety of tumors are neoplastic myoepithelial cells.

In metaplastic breast neoplasms consisting of mixtures of epithelial and mesenchymal elements, the cells with a mesenchymal appearance have an epithelial derivation, as shown by the presence of epithelial cell markers (e.g. cytokeratin positivity) and absence of mesenchymal cell markers [37, 38]. Epithelial–mesenchymal transition (EMT) is the process of disaggregating polarized epithelial units into single-motile fibroblastoid cells to enable cell movement and morphogenesis [39]. The process of EMT represents a potential mechanism for the progression of metaplastic malignancy, which is accompanied by loss of proteins associated with the epithelial phenotype (for example, E-cadherin and cytokeratin) and *de novo* synthesis of proteins associated with mesenchymal morphology (for example, vimentin). Transcriptional profiling revealed that many of the discriminator genetic profiles of metaplastic carcinoma were associated with down-regulation of epithelial phenotypes and with synthesis, remodeling and adhesion of extracellular matrix [32].

A monoclonal origin in metaplastic carcinoma has been suggested. The cellular precursors to metaplastic breast carcinomas may reside within the CD10(+) cell population, as transformation of these cells results in the development of rare claudin-low metaplastic tumors [19]. Moreover, CD10(+) breast cells with metaplastic traits can give rise to skin and epidermal tissues. These findings reveal the existence of a population of cells with epidermal progenitor activity within adult human breast tissues. However, it remains unknown whether all metaplastic breast carcinomas are derived from the same cellular precursors or whether different cell types contribute to the heterogeneity. Meanwhile, the theory of dedifferentiation [from well differentiated to poorly differentiated tumors] is challenged by genetic studies [40].

Pathogenesis of metaplastic breast carcinoma

There is limited information on the molecular etiology and signaling pathways involved in the pathogenesis of metaplastic carcinomas. A study by Hayes *et al.* concluded that activation of the *Wnt* signaling pathway is common in metaplastic breast carcinoma [41]. *CTNNB1* (β-catenin), *APC*, and *WISP3* gene mutations were seen in 41% of patients with metaplastic carcinomas. β-Catenin plays important roles in mammary development [42]. At the plasma membrane, β-catenin maintains mammary epithelial integrity. In the nucleus, β-catenin regulates gene expression programs that are essential for mammary development. Loss of β-catenin from cellular adhesive junctions and accumulation of this protein in nucleus predispose the breast to cancer development.

By immunohistochemistry, aberrant β-catenin accumulation was revealed in 92% metaplastic breast cancer cases [41]. Mutations in β-catenin are uncommon in common breast cancers. However, 25.9% of patients with metaplastic breast carcinoma had *CTNNB1* missense mutations within the NH_2-terminal domain, rendering the mutant protein resistant

to degradation [41]. Loss of PTEN and p53 tumor suppressors has been linked to the induction of β-catenin in breast cancer. In metaplastic breast tumors, PTEN expression is significantly down-regulated [18]. Additionally, p63, a member of the p53 gene family, has been shown to be highly up-regulated in metaplastic breast carcinomas [22]. The p63 isoform ΔNp63 interacts with protein phosphatase 2A, which inhibits glycogen synthase kinase-3β leading to β-catenin stabilization [43].

Wnt signaling plays a central role in mammary stem cell homeostasis and in breast cancer development. Over-expression of several members of the Wnt signaling cascade, including the Wnt1 ligand, β-catenin and mutant APC, results in the development of mammary tumors in mice that are reminiscent of metaplastic breast carcinomas in human [44-47]. The histology of these mammary tumors is highly heterogeneous, with areas of squamous, spindle cell and/or mesenchymal phenotype. In view of these mice genetic studies, the results indicate that constitutive activation of the Wnt/β-catenin signaling pathway expands the subpopulation of multi-potential stem/progenitor cancer cells. Expression profiles of these stem cells are more similar to tumor cells than to their own differentiated progenies [44], indicating that constitutive activation of Wnt signaling reactivates developmental pathways in mammary tissues, leading to metaplastic carcinomas.

P63 as a contributing factor in metaplastic carcinoma development

The sensitive and specific myoepithelial marker p63 plays a critical role in ectodermal differentiation during development and stratified epithelial progenitor-cell maintenance [48]. Unlike p53, whose protein expression is not readily detectable in epithelial cells, p63 is consistently expressed in basal cells of several types of multilayered epithelia and in myoepithelial cells of the human breast [49, 50]. It is a signature marker of stem cells during the development of mammary gland [50]. In contrast to the tumor suppressive function of p53, over-expression of p63 variants is observed in many squamous carcinomas suggesting that p63 can act as an oncogene [51]. P63 protein exists as two main isoforms, TAp63 and ΔNp63, with distinct, often opposite functions. Proteins with the transactivation domain are termed TAp63 and proteins lacking the transactivation domain are termed ΔNp63 [52]. TAp63-mediated transactivation of genes, such as Jagged-1 and EphA2 tyrosine kinase, promotes epidermal differentiation and development. TAp63 also downregulates EGFR expression, which is required for epidermal differentiation. By transcriptional inhibition of p53 target genes, ΔNp63 keeps epidermal cells in a proliferative state and inhibit terminal differentiation [53]. In adult mouse and human basal epithelial cells, ΔNp63α is the predominant p63 variant expressed at the protein level [54].

Breast cancers arising in carriers of germline BRCA1 mutations frequently have a basal-like phenotype. Conditional knockout of BRCA1 by transgenic expression of Cre recombinase in mice mammary gland leads to tumors that are characterized by high histological grade, central necrotic areas, and presence of metaplastic elements consist of neoplastic spindle cells or squamous cell differentiation in the form of keratin pearls or individual cell keratinization [55, 56]. Transcriptional upregulation of ΔNp63 proteins is critical for BRCA1 suppressor function [55]. BRCA1 is localized to a conserved intronic enhancer region within the Notch ligand Jagged-1 gene, an event requiring ΔNp63 for the normal differentiation process in breast tissue [57]. Defects in BRCA1-ΔNp63 signaling are key events in the pathogenesis of basal-like breast cancer, via transcriptional activation of Notch signaling pathway.

Taken together, p63 plays an important role in the regulation of breast stem/precursor cells. High p63 expression is closely associated with the development of metaplastic breast carcinoma. However, further explorations are necessary to reveal the role of p63 in regulating stemness associated with metaplastic tumorigenesis.

Prospective therapeutic development in metaplastic carcinoma

The World Health Organization recognized metaplastic breast carcinoma as a distinct pathological entity only in 2000. Currently, metastatic breast cancer is the chief cause of cancer-related death among women in the Western world. The increased incidence may represent an actual increase in the disease, or may be a result of improved awareness

and recognition by pathologists. However, due to its rarity and heterogeneity, there is no “standard” therapy for metaplastic breast carcinomas. Traditional chemo- and hormonal therapies for IDC are ineffective against metastatic breast cancers and are often associated with poorer survival. The gene encoding DNA topoisomerase II alpha, a molecular target of anthracyclines, is significantly down-regulated in patients with metaplastic breast carcinoma compared to those with basal-like IDCs, which may explain their poor responses to chemotherapy [18]. Since metaplastic breast carcinomas show stem cell-like features and high expression of genes related to myoepithelial differentiation and EMT [58, 59], blockade of the transition from epithelial to mesenchymal phenotype and/or tumor stem cell function may improve patient outcome. Human pathological studies as well as animal genetic studies suggest that activation of the Wnt signaling pathway is common in metaplastic carcinomas and that approximately 70% of these tumors show *EGFR* gene amplification and overexpression. These information may have treatment implications especially leading to targeted treatment for patients with metaplastic carcinomas. Other strategies have emerged to target the non-epithelial component of metaplastic mammary tumors by the combined use of ifosfamide with other agents, including etoposide or doxorubicin [60, 61]. In mice, mammary production of prolactin decreases the latency of tumors in the absence of p53, and increases the proportion of triple-negative claudin-low carcinomas, which display similarities to human metaplastic carcinomas [62]. The strong and selective presence of prolactin in the metaplastic cells may elicit growth stimulating effect on the breast epithelium [63]. In summary, these findings suggest that novel chemotherapeutic strategies guided by specific histology or targeted therapies based on individualized gene profiling are promising for the future.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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