Lymph Nodes in Breast Cancer – What Can We Learn from Translational Research?

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Summary

Clinical observations about lack of survival benefit after extensive axillary surgery and biological discordance between primary breast tumors and axillary lymph nodes raise the question of the actual metastatic potential of axillary nodal disease. The exploration of intratumoral heterogeneity and detection of genomic differences between the primary and lymph nodes indicate some similarity between the number of mutations in synchronous axillary node metastases and those in the primary lesion, suggesting a favorable prognosis. The hematogenous route of metastasis needs to be considered in findings of different subclones between nodal and distant metastases. Modern tools such as whole-genome sequencing applied in multiple tumor areas may guide more precisely the extent of axillary surgery.

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Introduction

Axillary nodal status traditionally represents the most important prognostic factor in breast cancer and more recently has become an important predictive tool in the age of genomic and per-

sonalized medicine to adapt systemic therapies accordingly [1]. Local axillary treatment was minimized with the introduction of the sentinel lymph node (SLN), revealing recurrence rates of up to 1% [2–6] and non-inferior survival compared to axillary lymph node dissection (ALND). More recently, the results from the ACOSOG Z0011 study indicated after a median follow-up of 9.3 years no survival benefit from ALND even when 1 or 2 SLNs were positive in clinically node-negative patients who had breast-conserving treatment, adjuvant radiotherapy, and in the majority of cases systemic therapy [7]. Also, historical data from the NSABP-04 study showed that only half of the expected patients with more advanced disease and approximately 40% having positive axillary nodes and no systemic treatment had developed an axillary failure after 25 years of follow-up [8]. Classical clinicopathologic predictors for nodal metastasis include young age, large tumors, high differentiation grade, and lymphovascular invasion. Transcriptional signatures have been developed for commercially available molecular assays to classify breast cancer more precisely and to guide postoperative systemic treatment more accurately [9]. However, molecular breast tumor subtypes are not necessarily concordant with axillary node subtypes. Furthermore, intratumoral heterogeneity needs to be considered in precision medicine.

Raising the question whether all axillary nodal disease has the potential to metastasize to distant organs (to guide the extent of local treatment) and whether systemic treatment plays a role in eradicating residual axillary disease, thus minimizing the probability of relapse, we here focus on genomic alterations and their impact on prognostic and predictive factors for the better understanding of axillary nodal disease.

Tumor Clonality – Intratumoral Heterogeneity

Intratumoral heterogeneity is defined as the presence of multiple genetically different cell populations within one tumor. Genomic characterization of metastatic disease when matched to the primary breast cancer has been shown to provide valuable information about clonality, acquired mutations, and metastatic potential [10–12]. Monoclonal and polyclonal seeding from the primary breast cancer to distant metastases has been observed in luminal subtype, polyclonal seeding in triple-negative basal and human epidermal growth factor receptor 2 (HER2)-enriched cancers. Polyclonal seeding is a mechanism to preserve heterogeneity in new distant metastases, maximizing the probability of drug-resistant clones. On average 55% of the primary mutations are retained in distant metastases.

In the metastatic progression models (linear and parallel), it is assumed that both primary tumor and metastases derive from one common progenitor cell and therefore are clonally related. In the linear progression model, the genetic discordance between primary and metastases is small in contrast to the parallel model where clone or subclone disseminate from the primary early resulting in a parallel genetic evolution and dissemination. Genetic alterations may also determine the route of seeding (lymphatic or hematogenous) and the organ tropism of a tumor (metastasis in particular organs).

Hypothetic metastatic scenarios [13] describe i) the same subclone of the primary tumor seeds in both the regional lymph node and the distant organ in a linear or parallel fashion; ii) different subclones from the primary tumor seed in the lymph node and the distant organ; iii) the same subclone seeds multiple metastases in different sites which may have the same clone or subclonal mutations (monophyletic metastases); iv) different subclones from the primary tumor seed in different sites in parallel (polyphyletic metastases); v) metastatic cells infiltrate the primary tumor (self-seeding) [14]; and vi) treatment-resistant subclones infiltrate treatment-sensitive metastases (cross-metastasis seeding) [15].

Concordance of Tumor Biology in the Primary Tumor versus Node Metastasis

Biological markers such as estrogen receptor (ER), progesterone receptor, and HER2 may be inconsistent during tumor progression [16]. In a retrospective study, Sun et al. [17] found that 38% of luminal A primary breast tumors switched to non-luminal A in the lymph node metastasis. The inconsistency in the biological subtype correlated with the intermediate-risk and the high-risk Oncotype DX® (Genomic Health Inc., Redwood City, CA, USA) Recurrence Score.

Bao et al. [18] investigated the molecular mechanisms of lymph node metastasis in breast cancer patients with and without sentinel lymph node metastasis. Whole-genome amplification and whole-exome sequencing were performed on different areas of the ER-positive primary and the SLN. One important finding indicated that different subclones coexisted in different areas of the primary, thus confirming tumor heterogeneity on the genomic level. Single-

cell sequencing identified a clonal mutation in the SLN with additional copy number changes (which was also found at low frequency) deriving from the primary tumor. Also, single cancer cells acquiring genetic alterations were found in the SLN suggesting the probability of distant dissemination. Assessing which aberrations were associated with the development of node metastasis, the authors found that *MCL1* (myeloid cell leukemia sequence 1) was more frequently altered in primary breast cancer with node metastasis versus no metastasis (high-level amplifications). Addressing the molecular profile of metastasis, no specific driver genes increasing the risk for metastasis in the nodes were found in a single-cell sequencing study. Similarly, Blighe et al. [19] found only few mutations private to the metastatic node. Ullah et al. [20] demonstrated that synchronous node metastases did not seed distant metastasis since no subclones were shared among nodal and distant metastases, thus suggesting a hematogenous route of metastasis.

Therefore, it is not clear if removal of metastatic lymph nodes has an impact on distant metastasis. Yates et al. [12] found that lymph node metastases from breast cancer had only a few mutated driver genes, but additional acquired mutations were found in distant metastases. Their findings support previous investigations showing that the number of mutations in synchronous axillary node metastases are very similar to the mutations of the primary lesion, indicating a chronologic probability of mutational accrual in comparison to distant metastasis with additional acquired mutations. Metachronous metastases show on average 63% more mutations than the primary tumor. The lack of new signatures in relapse after multiple lines of chemotherapy suggests that these agents are not drivers of mutations.

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform), GATA3 (transcription factor encoded by the *GATA3* gene), and MAPK (mitogen-activated protein kinase) pathway mutations are characteristic driver mutations in ER-positive primary breast cancer, TP53 (tumorsuppressor protein) and copy alterations are typical for triple-negative breast cancer. However, the mutational landscape changes in metastatic disease with TP53 mutations seen in 40–50% of ER-positive and increased PIK3CA, GATA3, and MAPK pathway mutations in ER-negative metastatic disease.

Models for Prediction of Nodal Metastasis

Gene expression profiling of the SLN was performed by Liang et al. [21] in a small study to predict non-SLN status using next generation sequencing. They identified 103 specifically expressed genes in the non-SLN-negative group and 47 in the non-SLN-positive group. Only 2 protein-encoding genes were identified with high expression levels.

A gene signature to characterize the metastatic microenvironment was developed using microarrays. A comparison of gene expression patterns between metastatic and non-metastatic lymph nodes of the same patient revealed different expression patterns. In particular, decreased expression of genes associated with immune response and

Fig. 1. Prediction test development.

overexpression of proliferation-associated genes was observed. Since gene expression changes were not seen throughout the axilla but in a node-to-node fashion, the authors suggested a more conservative surgical approach in the axilla [22]. Park et al. [23] used fluorescent in situ hybridization amplification and immunohistochemistry overexpression data from locally advanced breast cancer in the neoadjuvant setting. They found both overexpression of *WDR1* (protein encoded by the *WDR1* gen) and p-ERK (phosphorylated extracellular signal-regulated kinase) in the primary breast cancer and concluded a possible role in the nodal signature, in particular pN2–3.

Systemic Treatment-Associated Genomic Changes

A number of studies have shown extensive genomic heterogeneity within primary breast tumors and subclonal changes during systemic therapy. Primary systemic treatment may increase or decrease clonality in both the primary and metastases. Also the type of systemic treatment and cytotoxic drugs may impact differently on genomic changes. Thus, the identification of genetic variations that apply to the general population might be limited.

Many mutations are newly detected or enriched post-treatment as shown in ER-positive breast cancers. A high clonal instability may be due to selective proliferation of a subclone during estrogen deprivation [24].

Gellert et al. [25] evaluated the genomic alterations in ER-positive tumors treated with an aromatase inhibitor (AI) starting 2 weeks before surgery. Poor responders had an increased mutational load. *TP53*-mutated tumors were associated with poor response to AI, revealing reduced suppression of Ki67.

In patients treated with chemotherapy, tumors show a reduced mutation frequency. Variations in mutation status pre- and post-treatment are observed in about 24% of patients.

In some samples, new mutations were detected post-treatment, and some samples retained the same mutations after chemotherapy. Prospective trials in the neoadjuvant setting, such as GeparQuattro, GeparQuinto, and GeparSixto, as well as the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (Neo-ALTTO) study, show that patients with tumors with a *PIK3CA* mutation had decreased pathologic complete responses compared to those with wild-type *PIK3CA*, indicating a poorer prognosis [26].

These findings were confirmed recently by data indicating that loss of somatic mutations in *TP53* and *PIK3CA* may be translated into biomarkers for prognosis. However, molecular subtype was not significantly associated with changes in *TP53* and *PIK3CA* mutations [27].

Genomic Test for Prediction of Nodal Response to Treatment

A genomic test was developed at the MD Anderson Cancer Center to predict pathologic response (and not resistance) to sequential taxane-anthracycline-based chemotherapy as neoadjuvant treatment. This test was evaluated in a validation cohort, and the predicted sensitivity to chemotherapy in 28% of patients was associated with a pathologic response in 56% and significantly improved distant relapse-free survival (DRFS; 5-year DRFS 93%). Of particular interest was a significantly higher probability of negative pathologic nodal status after neoadjuvant chemotherapy in patients whose breast cancer was predicted to be sensitive to chemotherapy.

Overall, in a cohort of 153 patients, 65 (42%) were clinically node-negative (cN0) before treatment, of which 69% had a pathologic node-negative (pN0) status after completion of neoadjuvant

Fig. 2. Design of the SENTINA trial.

chemotherapy. In addition, 44% of clinically node-positive (cN+) patients at initial diagnosis converted to a pN0 status after completion of neoadjuvant chemotherapy.

In the genomic test, it was observed that 27 of 88 patients with cN+ status at initial diagnosis were predicted to have chemosensitive breast cancer, and these patients had a significantly higher probability of conversion to pN0 status after neoadjuvant chemotherapy (70%, 95% confidence interval (CI) 50–86) compared to those who were predicted not to be sensitive to chemotherapy (33%, 95% CI 21–46) (fig. 1). Also, 14 of 65 patients with cN0 status at initial diagnosis were predicted to have chemosensitive breast cancer, and 86% (95% CI 57–98) of those had pN0 status after neoadjuvant chemotherapy compared to 65% (95% CI 50–78) predicted not to be sensitive to chemotherapy. These preliminary data indicate that the probability of pN0 status after neoadjuvant chemotherapy is high in patients with predicted tumoral chemosensitivity, but there is no information on the accuracy of a sentinel lymph node biopsy (SLNB) procedure in this context. These preliminary results provide a strong rationale for additional research to determine whether the probability of achieving pN0 status, and of achieving negative non-SLNs, is sufficiently high for patients whose breast cancer is predicted to be chemosensitive to support offering SLNB after neoadjuvant chemotherapy using sequential 3-weekly anthracycline- and taxane-based treatments.

AGO-35 is a prospective, multicenter neoadjuvant study which aims to determine the combined accuracy of the genomic prediction of tumoral chemosensitivity and SLNB after neoadjuvant chemo-

therapy. Eligibility criteria include patients scheduled for anthracycline- and taxane-based neoadjuvant chemotherapy with HER2-negative tumors. Transcriptional profiling with Affymetrix U133A gene chips (Thermo Fisher Scientific, Waltham, MA, USA) and calculation of the pharmacogenomic prediction results will be applied on samples from routine breast needle biopsy at diagnosis. In cN+ patients, SLNB followed by axillary dissection will be performed after completion of neoadjuvant chemotherapy to evaluate the frequency of true-negative SLNB (i.e., the probability that all lymph nodes are negative if the SLNs are negative) according to clinical axillary lymph node status and predicted chemosensitivity [28, 29].

This prospective study would enable us to determine the combined accuracy of genomic prediction of tumoral chemosensitivity and SLNB after neoadjuvant chemotherapy to identify which patients could safely undergo conservative surgical management of their regional lymph nodes.

Clinical/Pathologic Models for Prediction of Lymph Node Metastases

Valid prediction of positive/negative axillary status after neoadjuvant systemic therapy might allow to tailor axillary surgery with the aim to limit extensive surgery to those patients with involved axillary nodes. In the setting of breast cancer patients who undergo primary surgery, several nomograms have been developed to predict the probability of non-SLN involvement in the case of a posi-

tive SLN. A validation of all models with the goal to compare their predictive capacity in a cohort of 561 patients with positive SLN suggests differences in model performance particularly among the subgroup of patients with limited axillary disease burden (micro-metastasis or isolated tumor cells) [30]. Patients from the SENTINA trial (fig. 2) were analyzed with the goal to validate 6 nomograms from the primary operative setting and 1 nomogram from the neoadjuvant setting in our neoadjuvant cohort. Among 181 patients in SENTINA converting from cN1 prior to neoadjuvant systemic therapy to ycN0 but found to have a histologically positive SLN, we found adequate area under the curve (AUC) values (0.82–0.71) when analyzing models derived from the primary operative setting. The neoadjuvant model yielded an AUC value of 0.66 which was inferior but within the published CIs. A newly developed ‘SENTINA nomogram’ (comprising the diameter of the largest lymph node ($p = 0.006$, odds ratio (OR) = 1.19), tumor size prior to neoadjuvant systemic therapy ($p = 0.085$, OR = 1.31), and number of all positive SLNs ($p = 0.083$, OR = 2.04) yielded AUC values comparable to previous nomograms [31]. In a second analysis, a de-novo nomogram was developed to predict ypN+ after neoadjuvant systemic therapy based on clinical/pathologic parameters. The performance of this nomogram was compared to the performance of individual clinical/pathologic parameters. This prediction model included parameters evaluable before/after definitive surgery (including ER, multifocality, lymphovascular invasion, detection of SLN after neoadjuvant systemic therapy, and ycT) and demonstrated acceptable accuracy. The limitation to parameters evaluable before surgery (i.e., ER, ycT) showed reduced accuracy which was comparable/superior to that of using individual parameters. Since tumor biology was the strongest parameter in our models, it may be hypothesized that modern tumor biological parameters might optimize the prediction of the axilla [32].

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Conclusion

In precision surgical oncology, the distinction between nodal metastasis with and without the ability to seed distant metastases may need to be further explored by genomic research. The genomic properties of ipsilateral synchronous axillary metastases are similar to those of the primary breast cancer. The detection of the metastasis-driving subclone in the primary tumor would enhance targeted systemic treatment and would enable omission of SLNB. Early (clonal) driver genes are shared between the primary and metastases, in contrast to late (subclonal) driver genes. Considering intra-tumoral heterogeneity, whole-genome sequencing provides more accurate information than other tools such as whole-exome sequencing which examines only parts of the genes. Technical aspects encompass the analysis of multiple tumor samples and tracking of genomic evolution in post-treatment samples to assess drug-resistant clones in a more complete genomic landscape. Investigation of genetic differences and similarities in primary and metastatic disease may support the prevention of metastatic disease in some tumors once there is a long time period between the emergence of the progenitor cell and clone and the metastasis.

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Disclosure Statement

The authors declare no conflict of interest.

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