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Heterogeneity of Drug Target Expression Among Metastatic Lesions: Lessons from a Breast Cancer Autopsy Program

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The target tissue for most cancer therapeutics is metastatic disease, either the treatment of overt metastases or the prevention of colonization by micrometastases in the adjuvant setting. Despite this, few drugs are tested in the metastatic setting in preclinical experiments, and relatively little is known about the expression of therapeutic targets in human metastases. A firestorm was ignited by several reports in which paired primary breast tumors and metastases were profiled by gene expression microarrays. Paired samples clustered together using multiple bioinformatic methods, suggesting little variation in gene expression patterns (1-3). Other reports using the same type of analysis identified alterations in gene expression between paired primary tumors and either lymph node or distant metastases (4-8). These reports are supplemented by studies at the protein level indicating a myriad of expression differences between primary tumors and matched metastases (rev. in (9), Supplemental Table 1). Whether there is a difference in molecular profile between primary tumors and their metastasis is not strictly an academic question, considering the fact that there is a growing belief that molecular target expression data should be used to enter patients onto clinical trials. Is the information provided by a primary tumor alone sufficient?

Wu et al. (10) recently analyzed tissues from rapid autopsies of ten breast cancer patients, providing a wealth of data on therapeutic targets in the metastatic setting. The patients were roughly half lymph node positive versus negative at diagnosis; the mean interval between diagnosis and death was 6.3 years (range 2-11). Metastatic tissue was harvested from 4-7 independent lesions, which represents an advantage over most other studies which have compared a primary with a single matched metastatic lesion. Tissues were obtained within four hours of death and should also be suitable for high quality RNA expression profiling.

Several trends observed in the expression of therapeutic targets are diagrammed on Figure 1. With regard to estrogen receptor (ER), six of the primary tumors were positive; the remainder of the cases met criteria for “triple negative” breast cancer. For the ER+ cases, the ER staining of the metastases was equivalent to that of the primary tumor in two cases, reduced (in terms of percentage of positive staining tumor cells) in two cases and negative in the remaining two cases. Similar trends have been reported previously in the literature, based on IHC analyses of primary tumors compared with a lymph node metastasis, in some (11-14) but not all (15,16) papers. Progesterone receptor (PR) expression followed similar trends in the autopsy study, decreasing in the metastases from two cases and becoming negative in an additional two cases. While many elegant pathways have been described for tamoxifen resistance, simple loss of hormone receptor expression must be considered another contributing factor.

The expression of several receptor tyrosine kinases was determined (10). Four of the ER- primary tumors were positive for EGF receptor (EGFR) by IHC. In each case heterogeneity

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was observed in EGFR levels among the metastases. Similarly, c-Met was expressed by IHC in the four triple-negative cases. c-Met staining was consistent between primary tumors and metastases in two of these cases and heterogeneous in the remaining two cases, varying from 0-2+ in the metastases. Her-2 was unamplified in all primary tumors, thus limiting the analysis of this receptor tyrosine kinase. FISH data for the metastatic tissues was unamplified in the vast majority of cases, but reached 3.0 in an omental sample from a single case.

Wu et al (10) also presented a comprehensive analysis of the DNA methylation status of seven gene promoters. Relative concordance of promoter methylation status among primary tumors and multiple metastases was found for the *HIN1*, *Twist*, *estrogen receptor-α (ER)* genes, while various degrees of discordance was found for *RASSF1A*, *Cyclin D2*, *APCc1* and *RARβ* (Figure 1). The data suggest that some genes may be more amenable to epigenetically based therapies than others.

While the complete loss of a therapeutic target in a metastatic lesion makes sense as a potential determinant of drug response, what is the significance of a change from 2+ to 1+ intensity, for example? Few targets have been comprehensively examined for the relationship of quantitative expression to drug efficacy, yet the idea is attractive. One of the best examples is ER, where a quantitative “Allred score” has been developed. The “Allred score” reflects the percentage of positively staining tumor cells added to an average intensity score. This continuous score segregated patient disease free survival to endocrine therapy, suggesting that intervals in expression may be biologically meaningful (17). In a neoadjuvant chemotherapy trial, the Allred score increased between biopsy and surgery in 49/296 breast cancer patients and was associated with improved DFS but not overall survival (18). When ER was quantified by another multi-level algorithm, a shorter time to recurrence was associated with lower levels of ER in tamoxifen (P=0.07) and anastrozole (P=0.009) treated patients in the ATAC trial (19). For EGFR, multiple metrics of expression have been imperfectly correlated with therapeutic response, including protein expression, mutation and gene amplification. Using IHC, EGFR expression levels in lung cancer have been quantified as either positive or negative, and conflicting correlations obtained with response to kinase inhibitors (20). It will be of interest to determine the impact of target expression level, and heterogeneity of expression among lesions, on the efficacy of this and other therapeutic targets. Support for autopsy studies where samples can be analyzed from multiple metastases has been limited in recent years, but may be a worthy adjunct to preclinical validation studies.

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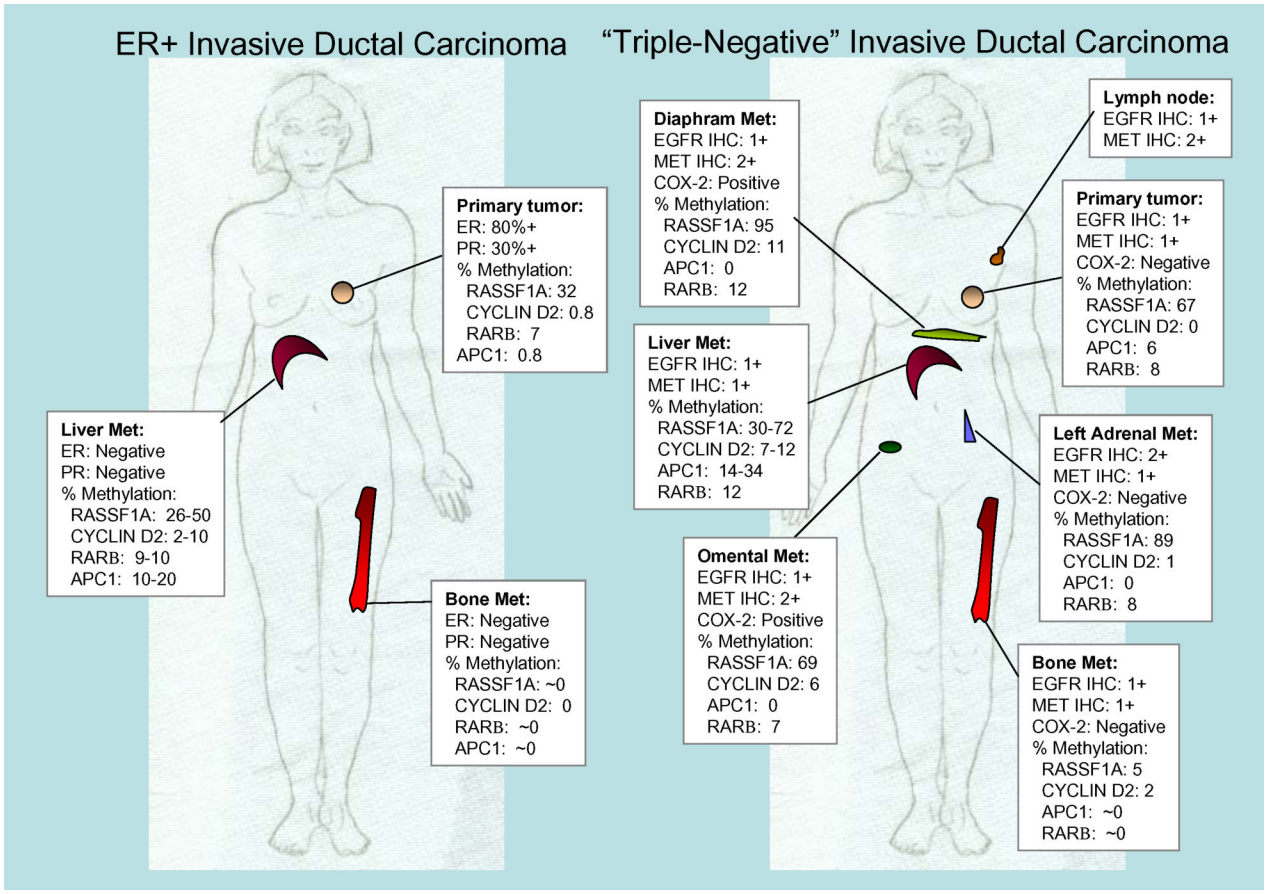

Figure 1.

Representative Cases from a Rapid Autopsy Program (10). Metastases were harvested within four hours of death from ten patients and compared to the matched primary tumor. The results from two cases are diagrammed. IHC staining for estrogen receptor (ER) and progesterone receptor (PR) is based on the percentage of positive cells; that for EGF Receptor (EGFR) and c-Met (MET) is on a 0-3+ intensity scale; and Cox-2 is positive/negative. Percentage DNA methylation of gene promoters was determined by QM-MSP assay. Methylation of the *HIN1*, *Twist* and *ERα* genes was relatively uniform in all samples.