Lessons from an Aggressive Cancer: Evolutionary Dynamics in Esophageal Carcinoma

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Abstract

Rapid progression to metastatic disease and an intrinsic resistance to any type of systemic therapy are hallmarks of aggressive solid cancers. The molecular basis for this phenotype is not clear. A detailed study of the somatic progression from local to early systemic esophageal cancer revealed rapid diversification of cancer cells isolated from various sites, but also evidence for early clonal expansion. These findings have implications for diagnostic pathology and therapeutic decision making. [Cancer Res 2009;69(13):5285–8]

Background

Despite complete curative resection of the primary tumor and adjuvant systemic therapies, 10% to 95% of patients will die of systemic cancer—depending on tumor (subtype). The reason for this enormous variation in aggressiveness is largely unclear. It was noted that poorly differentiated tumors overexpress genes normally enriched in embryonic stem cells and thereby differ from well-differentiated tumors (1). These stemness-associated pathways are thought to explain the tumor-propagating potential of several cancers. However, the reason why tumors of comparable grading but from different tissues display varying levels of aggressiveness may be linked to other properties including the type of genetic damage or the abilities to spread or colonize. Because we could recently show that tumor cell dissemination is an early event even for the less aggressive cancers arising in breast and prostate tissue (2, 3), the time point of dissemination is an unlikely cause for differences in outcome. Although cancer is often viewed as a two-stage disease, the first being a locally restricted stage (stage N0, M0), the second a manifestation of regional or distant spread (stage N1, M1), this digital thinking might be misleading. The true nature of the disease might be better conceptualized within an evolutionary model, in which the continuous selection of genetically unstable variant cells and their expansion determines disease course and risk of dying from cancer. In this evolutionary model it is not a question of a sudden onset of metastatic dissemination with short subsequent latency until metastatic manifestation is central, but rather a dynamic process of mutation, selection, clonal expansion, and genetic diversification taking place simultaneously at various sites within primary and secondary sites (4). Such a parallel nature of local and systemic disease might in particular underlie rapidly deteriorating and aggressive cancers, one of which is esophageal cancer. Hence, an analysis of the systemic progression of this cancer might add to our understanding of malignant behavior.

We therefore studied the early phase of disseminated disease in esophageal cancer. Both esophageal carcinoma subtypes, squamous cell carcinoma and adenocarcinoma, are characterized by early tumor cell dissemination and metastasis, resulting in equally poor survival in both entities. Moreover, they display a similar metastatic pattern, although being quite different with respect to their etiology and biology (5–7). In expert centers 5-year survival for patients treated with surgery alone is low, with survival for stages I (T1N0), IIA (T2–T3N0), IIB (T1–T2N1), and III (T3–T4N1) of 84%, 49%, 27%, and 17%, respectively (8, 9). It should be noted, however, that at the time of diagnosis only one third of patients will be considered operable, and that after surgical exploration, tumor resection will be possible in only 15% to 20% of patients. Therefore, the survival data above represent the minority of “good” prognosis patients (10). Neither neoadjuvant (systemic chemotherapy before “curative” surgery) nor adjuvant therapy (chemotherapy after “curative” surgery) have proven to be a substantial effect on survival (10). It was therefore concluded that local control can rarely be achieved and that esophageal cancer is characterized by early micrometastatic spread, which is highly insensitive to systemically acting drugs (11). Our current inability to eradicate such disseminated tumor cells (DTCs) before the manifestation of metastasis has been explained by different hypotheses including insensitivity of noncycling (dormant) DTCs or highly resistant (ref. 12) (stem cell-like?) DTCs. On the other hand it has been convincingly argued that DTCs should be ideal targets for systemic therapies because they reside as single cells or small colonies and therefore should be much more accessible for any type of drug than fully established metastases (13). Moreover, tumor load is minimal after surgery and therefore the number of resistant variant cells will never be smaller than in this particular clinical situation. Thus, one cannot escape the notion that the lack of success of adjuvant therapies is related to our lack of knowledge about systemic cancer progression in general and the target cells specifically.

This clinical situation, but also the unresolved questions about the metastatic process, prompted us to study the metastatic seed in operable esophageal cancer patients. The presence of latent cancer spread has been frequently assessed in esophageal cancer patients. Two organs were explored: lymph nodes and bone marrow. Detection of DTCs (either by immunohistochemistry or PCR) in patients staged as free of nodal metastasis by routine histology had prognostic impact in all (14–20) but two studies (21, 22). Three studies identified the presence of DTCs in lymph nodes as an independent risk factor (15, 23, 24). DTCs were also detected in bone marrow (12, 25–29), and the two studies that assessed the prognostic impact found a strong association with poor outcome (26, 29). The presence of DTCs in two different organs, representing the two major routes of metastasis (lymphatic and hematogenous), allowed us to study and compare their genomic characteristics.
Key Findings

Clonal divergence of primary tumors and DTCs. We isolated DTCs from lymph node and bone marrow samples from esophageal cancer patients in nonmetastatic disease (M0 stage) and did metaphase-based comparative genomic hybridization (CGH) after whole genome amplification (30). Primary tumors were laser-microdissected to ensure purity of epithelial cells for CGH. Unsupervised cluster analysis showed that DTCs and primary tumors display rather different chromosomal aberrations. This finding was not surprising, as it had been previously shown that esophageal adenocarcinoma and lymph node metastases diverge genetically (31). More interesting was the observation that HER2-gene amplification, which was the most frequent chromosomal alteration in DTCs from lymph nodes and bone marrow (see below), was not conserved between primary tumors and DTCs. Neither presence nor absence of HER2 gains in primary tumors was predictive for the HER2 status in DTCs of the same patient. However, HER2 was significantly more often gained in cells derived from esophageal adenocarcinomas than in cells derived from esophageal squamous cell carcinomas. When we tested the impact of the HER2 oncogene amplification in both subtypes on survival, the result was surprising: when primary tumor samples were analyzed, HER2 amplifications did not increase the risk for short-term survival ($P = 0.7$), whereas all patients displaying a gain of HER2 in single DTCs from bone marrow or lymph node died within 23 months ($P = 0.005$). Upon multivariate analysis only HER2 gain in DTCs and large primary tumor size were independent risk factors in this cohort of patients (30).

This observation has several implications. As we observed an HER2 gain in DTCs only in 40% of cases that displayed the HER2 gain in either primary tumor or in DTCs, it is safe to conclude that an HER2 gain can be acquired by DTCs after dissemination from the primary site. As some primary tumors displayed an HER2 gain whereas their DTCs were negative, the opposite is also correct: HER2 can be amplified in the primary tumor at a later stage and HER2-positive primary tumor cells do not necessarily disseminate at a detectable frequency. The independent acquisition of an HER2 gain in some cases of matched primary tumor-DTC pairs together with the differential prognostic impact of HER2-positive cells in bone marrow and lymph nodes versus primary tumors raised the question whether HER2 has a different role in primary tumors and DTCs. In patients in which DTCs with an HER2-gain spread throughout the body by the way of blood or lymph, these cells may contribute to particularly aggressive disease. Given its importance...
in systemic but not in local cancer, interference with the HER2 pathway might affect differentially cells from primary tumors and DTCs, especially in patients with esophageal adenocarcinoma. To test this, we studied the only available pair of cell lines that was generated from a primary tumor (PT1590) and from lymph node DTCs (LN1590) of a patient with esophageal adenocarcinoma (32). Both cell lines display an HER2 gene amplification and express similar amounts of the protein. However, after siRNA-mediated knockdown, only the DTC-derived cell line responded with growth reduction and apoptosis. Likewise, the HER2 inhibitors lapatinib and erlotinib reduced cell numbers and induced apoptosis only in LN1590 cells (30). Together, clinical and functional data support that primary tumors and DTCs diverge genetically and functionally and this divergence may contribute to the aggressive nature of the disease and the failure of adjuvant therapies.

**Clonal divergence of DTCs from different organs.** Because we were able to isolate DTCs from metastasis-free lymph nodes and from bone marrow for CGH analysis, we compared their genomes. Interestingly, the DTCs from both organs displayed different chromosomal aberrations. Striking disparity included chromosomes 7q, 10q, and 5q. In three patients we isolated several cells from both compartments and these also displayed different genetic changes. Thus, it seems that either dissemination to bone marrow and lymph nodes or, more likely, survival or expansion in the two organs requires a different set of chromosomal alterations. However, gain of HER2 was noted in both organs as the most frequent amplification in DTCs. This finding further supports a functional role of HER2 in systemic esophageal cancer.

**Clonal expansion at various sites as sign of aggressiveness.** Genetic instability generating variant cells will lead only to clonal diversity if the variant cells are viable and can expand. Expansion of diverse clones may then be a characteristic trait of aggressive cancer. We therefore checked all patient samples in which we had successfully isolated several DTCs from one organ. Genetic profiles from 32 cells isolated from lymph nodes or bone marrow of 12 patients were compared. The cells from one individual patient were genetically similar if isolated from the same organ, providing evidence for both clonal diversification between organs and of clonal expansion within one organ. This is in striking contrast to other cancers such as prostate cancer, which is characterized by relatively slow progression over a decade from local to metastatic disease. During the stage of nonmetastatic disease, prostate cancer cells are generally very heterogeneous and several DTCs often display completely different chromosomal gains and losses (3). However, once manifest metastasis is diagnosed, single DTCs from bone marrow of prostate cancer patients become genomically highly similar, providing firm evidence of clonal expansion of a very aggressive cell that has acquired important genomic rearrangements (3). It seems that the much faster generation of ectopically expanding tumor cells in esophageal cancer patients, in which these genotypes can already be found in M0 stage disease, is a hallmark of aggressive systemic progression (Fig. 1).

**Significance**

**Chromosomal aberrations: Markers of descent or of genome function?** Several conclusions can be drawn from these observations. Different genomes are selected in primary tumors, lymph nodes, bone marrow, and probably other ectopic sites (such as lung, liver, brain-organs inaccessible for ex vivo analysis in patients) precluding the use of chromosomal aberrations to assess clonal relationships, at least at the level of resolution provided by metaphase CGH. Important genetic aberrations will be selected outside the primary tumor and given the limited number of advantageous (i.e., selected, expanded, and driving) rearrangements and mutations that are observed in human cancers, the independently emerging genotypes may converge to some degree. These independently acquired chromosomal gains and losses may then fulfill different functions within a cell depending on the genetic or epigenetic context as observed for the diverging role of HER2 gains in primary tumors and DTCs of esophageal cancer patients. Clonal diversity, which, within the primary tumor, has recently been shown to be a greater risk factor than a homogenizing clonal expansion for esophageal adenocarcinoma in the study by Maley and coworkers (33), as opposed to cellular diversity, may be a characteristic of aggressive cancers when present at ectopic sites.

**Development of a novel diagnostic pathology.** These observations may pose obstacles as well as provide opportunities for therapeutic approaches. The identification of genetic changes driving clonal expansion may allow specific targeting in adjuvant therapy strategies. The clonal diversity may require several targeting strategies (4). In any case, a novel diagnostic pathology is needed that determines directly the (genetic) Achilles’ heels of esophageal DTCs. On the other hand, targeting less aggressive cancers that are characterized by cellular diversity without clonal expansion over prolonged periods of time such as prostate cancer may require rather different strategies. In both cancers, however, the primary tumor was found to be a poor surrogate marker for the genotypes of DTCs. It is, therefore, very likely that prediction of therapy response, in particular to targeted therapies, will require a detailed knowledge about the foe, the tumor cells that spread throughout the body.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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