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Review

# Turning the headlights on novel cancer biomarkers: Inspection of mechanics underlying intratumor heterogeneity

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ABSTRACT

Although the existence of intratumoral heterogeneity (ITH) in the expression of common biomarkers has been described by pathologists since the late 1890s, we have only recently begun to fathom the staggering extent and near ubiquity of this phenomenon. From the tumor's perspective, ITH provides a stabilizing diversity that allows for the evolution of aggressive cancer phenotypes. As the weight of the evidence correlating ITH to poor prognosis burgeons, it has become increasingly important to determine the mechanisms by which a tumor acquires ITH, find clinically-adaptable means to quantify ITH and design strategies to deal with the numerous profound clinical ramifications that ITH forces upon us. Elucidation of the drivers of ITH could enable development of novel biomarkers whose interrogation might permit quantitative evaluation of the ITH inherent in a tumor in order to predict the poor prognosis risk associated with that tumor. This review proposes centrosome amplification (CA), aided and abetted by centrosome clustering mechanisms, as a critical driver of chromosomal instability (CIN) that makes a key contribution to ITH generation. Herein we also evaluate how a tumor's inherent mitotic propensity, which reflects the cell cycling kinetics within the tumor's proliferative cells, functions as the indispensable engine underpinning CIN, and determines the rate of CIN. We thus expound how the forces of centrosome amplification and mitotic propensity collaborate to sculpt the genetic landscape of a tumor and spawn extensive subclonal diversity. As such, centrosome amplification and mitotic propensity profiles could serve as clinically facile and powerful prognostic biomarkers that would enable more accurate risk segmentation of patients and design of individualized therapies.



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## 1. Intratumoral heterogeneity: a tumor's much-desired destination

The last few decades of cancer research have yielded irrefutable evidence regarding the presence and strong clinical implications of interpatient and intrapatient tumor heterogeneity (Almendro et al., 2014a; Bedard et al., 2013; Navin et al., 2010; Yap et al., 2012). Theoretically tumors arise from a single transformed cell; however by the time of diagnosis, several genetically distinct populations of cells can be detected. This diversity within a tumor is referred to as intratumoral heterogeneity (ITH) (Maley et al., 2006; Park et al., 2010; Ye et al., 2009). Bewildering amounts of spatial and temporal ITH are now known to be present in several cancer types. In an analysis of 100 breast cancer genomes (Stephens et al., 2012), driver mutations were found in 40 different cancer genes in 73 different combinations. While 28% of these tumors had a single-driver mutation, the remainder had multiple alterations, with some tumors presenting with as many six driver mutations. Six driver mutations have never been intentionally targeted by clinicians at one time; this fact is certainly a wake-up call in terms of what a prohibitive challenge ITH poses for cancer treatment, especially when such treatment needs to be personalized for optimal outcomes. So profound is ITH in fact, that another breast cancer study in which single nuclei genome sequencing of 50 tumor cells was carried out, concluded very alarmingly, that "no two cancer cells within the same cancer have the same genome" (Wang et al., 2014). Moreover, ITH bedevils every stratum of the disease; within the primary tumor, between the primary and the metastatic lesion, and even between different metastases. Traditionally, clinical guidelines have focused on maximizing the patient populations that benefit from targeted therapies. In the face of incontrovertible evidence related to ITH, testing guidelines have been compelled to accommodate ITH. The 2013 ASCO/CAP guideline for breast cancer indicates that a tumor is HER2-positive if more than 10% of cells overexpress HER2. Additionally, the guideline states that heterogeneity should be reported (Wolff et al., 2013).

In recent years, viewing a tumor through the clarifying lens of ecological concepts has aided cancer biologists in developing frameworks for analyzing and understanding the dynamic population interactions that occur as ITH is generated (Cleary et al., 2014; Crespi and Summers, 2005); by focusing on "societal" relationships among cancer subpopulations, a better understanding of how these subpopulations can reciprocally influence each other's growth rate, metastasis, immune sensitivity, and therapeutic responses is gradually emerging. Among the fundamental interactions common to both diverse clonal sub populations and species in an ecosystem are competition and cooperation (Fig. 1A). Levels of competition and cooperation depend on a wide variety of factors including varying tumor microenvironments, genetic, epigenetic and proteomic differences between neighboring cells, as well as availability of resources and the fitness of those cells in the niches present. These complex features of ITH provide important insights into therapeutic challenges frequently encountered in the clinic particularly when clinicians are confronted with aggressive tumor phenotypes.

What is the significance of considering a tumor as a diverse ecosystem? Diversity has been known to stabilize an ecosystem in the face of selective pressures and environmental changes. Diverse ecosystems are more productive, filling available niches and using resources more effectively (Cardinale, 2011; Hautier et al., 2014; Hector, 2011). Stability, in an ecological context, entails remaining productive (*i.e.* producing biomass) despite challenges to the ecosystem (Bezemer and van der Putten, 2007). For example, if a prairie undergoes a drought, there will be a decline in the number of grass species that cannot withstand excessively dry conditions. However, those grasses that can withstand the harsh conditions will be more productive, resulting in very little change in overall ecosystem productivity and the continued efficient use of available resources. To draw a parallel with tumor biology, a particular species in an ecosystem would be analogous to a particular tumor cell clone, and different species would be equivalent to cancer cell subclones that have diverged genetically from their parents. A resistant tumor displays growth and stability, even in harsh conditions such as chemotherapy or radiation treatment, similar to a diverse prairie ecosystem.

In addition to facilitating the development of therapeutic resistance, diversity could also provide a multitude of benefits that might aid in metastasis, a key phenomenon underlying poor prognosis. While the prairie grass example earlier highlights adaptability of an ecosystem where the players involved are static, diversity within mobile populations can lead to successful migration out of crowded areas into new niches. These new environments can be physically distant or proximal with differing resources and conditions. It has long been posited that the process of metastasis exerts considerable selective pressure on the migrating tumor cells (Fidler and Hart, 1982). The larger the pool of diverse clones metastasizing from a tumor, the more likely one will not only survive the migration process but also be better adapted to a new site. As ITH develops, some clones will be less suited for the current environment but would be capable surviving the voyage to, and settlement of, a more suitable secondary site. In essence, high levels of ITH would allow for therapeutic resistance and