Preface

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Published online: 11 May 2021

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Intratumoral heterogeneity of primary tumors has long been recognized by clinical pathologists and is currently a focus for basic research. Building on the observed heterogeneity, tumor progression was suggested by Drs. Rous and Beard based on studies of virus-induced rabbit papillomas [1]. The observation that tumors, throughout their progression, have multiple cellular morphologies was reviewed by Dr. Foulds, who emphasized that different areas of tumors exhibited diverse, but uniform, histomorphologies [2]. Subsequent studies of tumor heterogeneity documented distinct differences in cellular morphology within the primary tumor, between the primary tumor and metastatic foci, and between metastatic foci supporting the concept of tumor progression. These observations provided the basis for the concept of tumor evolution, building on the phenotypic, molecular, spatial, and temporal changes in neoplasms during tumor progression and therapeutic treatment. Studies by Drs. Fidler and Kripke introduced the concept of metastatic heterogeneity within tumors, based on the observation that tumors contained cellular subpopulations with varying metastatic potential [3]. Subsequent preclinical studies from their laboratories demonstrated that metastatic variants were selected by the process of metastasis, introducing the concept of clonal selection, extending experimental [4] and spontaneous metastasis models [5]. Recently, single and multi-sample sequencing studies have defined the tumor mutational landscape and its evolutionary trajectory, demonstrating widespread genetic and transcriptomic heterogeneity in both spatial and temporal dimensions. These alterations, together with selection, increase the number of cancer cell subpopulations in response to tumor progression and therapy. Indeed, genetic and transcriptomic heterogeneity is crucial

for tumor growth, invasion, metastasis, and resistance to therapy, as it provides the cellular plasticity forming the base for cellular selection.

In the past decade, the phenotypic, genetic, transcriptomic, and functional heterogeneity of tumors has been extended to include antigenic and immunogenic properties, as well as differences in the frequency and phenotypes of tumor-infiltrating myeloid and lymphoid cells. These findings, combined with recent regulatory approval of novel forms of immunotherapy, resulted in the addition of immunotherapy as a standard of care modality for a limited number of malignant pathologies, stimulating intense interest in extending the identified responsive tumor pathologies. Taken together, these studies document that clonal diversity provides a fecund soil supporting tumor evolution, ultimately shaping genomic features, including the tumor mutational burden and karyotypic profile, as well as the tumor antigenicity and immunogenicity. Ultimately, the transcriptomic, proteomic, phenotypic, genomic, immunologic, and functional heterogeneity, upon interactions with the tumor microenvironment (TME), including nutritional and immunological events, impact functional and immunological processes during tumor evolution. While molecularly targeted cytostatic and cytotoxic intervention and development of multidrug resistance is a well-established concept, only recently has this selective process been extended to host-associated immune regulation of adaptive T cell responses. Host adaptive immunity can regulate the evolution of the tumor antigen landscape via immunoediting and clonal evolution, as dictated by immune cells. This process has been confirmed by multiregional sequencing studies of tumor biopsies, documenting spatial intratumoral heterogeneity of tumor-infiltrating lymphocytes (TILs) in correlation with local expression of neoantigens.

The development of neoantigens, against which cytotoxic T lymphocytes (CTL) may be effective, is associated in part with genomic instability, resulting in tumor cells that are prone to accumulating genetic alterations and therefore a major source for tumor antigenic heterogeneity. Microsatellite instability (MSI) favors the expansion or contraction of short, repeated DNA tracts downstream of defects in DNA mismatch repair (MMR). MSI has been associated with extreme

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heterogeneity in neoplasms and is generally coupled with abundant TIL infiltration and an improved prognosis. Indeed, MSI-high (MSI-H) tumors often present a hypermutator phenotype, increasing their likelihood of inducing neoantigens that can be recognized by host immunity and respond clinically to immune checkpoint inhibitors (ICIs). Extensive evidence supports the role of tumor heterogeneity in the likelihood that malignant cells will survive chemotherapy, radiation therapy, and targeted anticancer agents. Moreover, tumor heterogeneity has been shown to impact the efficacy of immunotherapy, in particular vaccines and ICIs, resulting in the selection of innate and acquired immunotherapy refractory variants. Tumors that have defects in DNA MMR are sensitive to ICIs, largely reflecting their neoantigen load and elevated lymphocyte infiltration. This observation is supported by the response to the program death protein-1 (PD-1) blocker, pembrolizumab, for the treatment of MSI-H tumors, irrespective of histological derivation. Nevertheless, not all MMR-deficient tumors respond to ICIs, and may acquire resistance. Indeed, both the degree of MSI and CTL infiltration can appear heterogeneous in MMR-deficient tumors, resulting in tumor regions with limited antigenicity, scarce TIL infiltration, and potential for ICI resistance.

In summary, the heterogeneous nature of neoplasms, with regard to a number of parameters, is recognized and has been documented to impact clinical, cytotoxic, and immunotherapeutic responses. Indeed, there is abundant evidence that elevated tumor heterogeneity negatively influences clinical responses to most forms of treatment, including immunotherapy, suggesting that combinatorial regimens are critical for the “cure” of malignant tumors. However, while targeting spatial heterogeneity in the malignant compartment of the TME may

be theoretically feasible, temporal heterogeneity will emerge unless tumors are fully and rapidly eradicated, controlling tumor progression. Determining the sources of tumor heterogeneity, its dynamics, and key spatiotemporal features is critical for the development of novel and effective therapeutics. Despite the initial enthusiasm for immunotherapy, it is clear that not all approaches will have a major impact on spatial and temporal tumor cell heterogeneity, impacting the degree and duration of a clinical response. Understanding these parameters within the TME, including responses to treatment, may support the characterization of novel therapeutic targets and critically, strategies to implement multimodal interventional strategies that can include multiple immunotherapeutic strategies.

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