CHAPTER

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Toward an Ultimate Explanation of Intratumor Heterogeneity

Frédéric Thomas[*], *Beata Ujvari*[**], *Cindy Gidoin*[*], *Aurélie Tasiemski*[†], *Paul W. Ewald*[‡], *Benjamin Roche*[*,§]

*MIVEGEC (Infectious Diseases and Vectors: Ecology, Genetics, Evolution and Control), UMR IRD/CNRS/UM 5290, Montpellier, France

**Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia

[†]Lille1 University, UMR CNRS 8198, Evolution, Ecology and Paleontology Unit, Villeneuve d'Ascq, France

[‡]Department of Biology and the Program on Disease Evolution, University of Louisville, Louisville, KY, United States

[§]UMMISCO (International Center for Mathematical and Computational Modeling of Complex Systems), UMI IRD/UPMC UMMISCO, Bondy, France

No biological problem is solved until both the proximate and the evolutionary causation has been elucidated. Furthermore, the study of evolutionary causes is as legitimate a part of biology as is the study of the usually physico-chemical proximate causes. ***Mayr (1982)***

It is well established that extensive variation that can be inherited across cell cycles (including genetic, cytogenetic, and epigenetic) and phenotypic diversity exist within tumors (i.e., intratumor heterogeneity, referred to as ITH hereafter) (Fidler, 1978; Heppner, 1984; Marusyk and Polyak, 2010; Marusyk et al., 2012; Yates and Campbell, 2012). The implications and clinical importance of ITH are considerable since it affects key oncogenic pathways, drives phenotypic changes over time, and also poses a significant challenge to cancer medicine, being the primary underlying cause of resistance to systemic therapies (McGranahan and Swanton, 2015; Merlo et al., 2006).

The origins of ITH have been the subject of much discussion by investigators from diverse fields but no consensus has emerged. From a mechanistic perspective, it has been suggested that genomic instability is *the* major process generating ITH (Burrell et al., 2013). Apart from this proximate explanation, several nonmutually exclusive models have been proposed to explain the establishment and the maintenance of ITH (Michor and Polyak, 2010). For instance, Waclaw et al. (2015) described a model for tumor evolution that shows how short-range migration and cell turnover can account for rapid cell mixing inside the tumor. The cancer stem cell hypothesis postulates that the differentiation of few cells with stem cell properties, notably unrestricted self-renewal abilities, generate various cell types in the tumor, leading to ITH (Campbell and Polyak, 2007). In parallel, the linear clonal evolution hypothesis suggests that the accumulation of various hereditary changes over time confer different selective advantages to premalignant and malignant cells, and hence gives rise to ITH (Gerlinger and Swanton, 2010). The plasticity cell hypothesis states that the majority of tumor cells have varying degrees of stem cell–like characteristics, depending on cell intrinsic stochasticity and/or microenvironmental conditions (Michor and Polyak, 2010). For instance, Lloyd et al. (2016) recently proposed that at least some intratumoral heterogeneity in the molecular properties of cancer cells is governed by predictable regional variations in environmental selection forces. Broadly, these hypotheses argue that due to ITH's key role in neoplasia, cancer progression, and therapeutic resistance, its persistence, once initiated, is supported by various selective benefits. It is apparent that ITH may

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contribute to the persistence and continued evolution of a cancer (e.g., through drug resistance, immune evasion, and metastasis); however, because environments change unpredictably and evolution cannot anticipate the future, it is challenging to explain the occurrence of ITH at the very first steps of tumorigenesis. Michor and Polyak (2010) proposed the importance of stem cells when discussing ITH in early stage of in situ cancers because only stem cells with unlimited self-renewal capability are able to persist over time and accumulate the genetic and epigenetic changes that will potentially be retained by selection. Conversely, Ling et al. (2015) recently argued that ITH is so extreme, even in tiny tumors, that it implies evolution under a "non-Darwinian mode" because genetic diversity observed would be orders of magnitude lower than predicted by simple classic Darwinian selection, suggesting that the observed diversity is much less structured than expected theoretically.

Here, we propose a novel parsimonious Darwinian scenario, nonmutually exclusive with the others, to explain elevated ITH not only in late but also in early stage tumors. We argue that such heterogeneity could be the result of a selective process, bet-hedging, that starts from the very first steps of oncogenesis for specific reasons. In evolutionary ecology, bet-hedging is defined as a strategy that reduces the temporal variance in an organism's fitness at the expense of lowered arithmetic mean fitness. We adapt this definition to oncogenesis by considering it to be a process that reduces the temporal variance in the success of a cancerous or precancerous cell relative to that of other cells in the organism. Success, being the analog of evolutionary fitness, takes into account both the reproduction and survival of the abnormal cells within the organism. In oncogenesis, selection for bet-hedging favors cells that are more prone to generate variation in their progeny. The most obvious source of this variation would be a proneness to mutation (e.g., a mutator phenotype), but other sources of variation, such as increased tendency to undergo epigenetic changes could also play a role.

FROM COOPERATION TO SELFISHNESS AND BACK TO (MALIGNANT) COOPERATION

Neoplasia originates from normal cells that lose their typical cooperative behavior, become malignant, and hence proliferate to greater numbers than would normal cells. Diversification of cellular activities and tissue development in solid tumors (e.g., angiogenesis) suggest that cancer cells increase in abundance in part by engaging in cooperative activities. At many levels of life organization, from plants to human societies, emergence of cooperation in communities of selfish individuals occurs under adverse environmental conditions (Andras et al., 2007; Nowak and Highfield, 2011). Similarly, genetically distinct tumor cells are able to cooperate via the exchange of different diffusible products to overcome host defenses and fluctuating microenvironmental challenges (Axelrod et al., 2006), providing malignant cells, that have both selfish and cooperative characteristics, with a selective advantage.

As solid tumors develop only in nonliquid tissues, we can hypothesize that competition with healthy cells and space constraints (which are greater in nonliquid tissues than in liquid environments) are the main microenvironmental factors that favor the selection of cooperative behaviors in malignant cells and lead to tumor formation.

DE NOVO TUMOR DEVELOPMENT—FROM AN INDIVIDUAL CELL TO A COOPERATING ORGANIZED SYSTEM

Solid cancers are not simply clones of cancerous cells; they are complex and well-organized systems that have been compared to functional, though abnormal, organs (Egeblad et al., 2010). Oncogenesis not only generates sophisticated levels of convergent organization within a few months or years (Chen and He, 2016), but also often does so de novo in most cancers; each cancer must reinvent the wheel because its evolutionary products will die with the host (Arnal et al., 2015) (see Chapter 12). In this context, bet-hedging appears to be a widespread mechanism to produce de novo such malignant, elaborate, cooperative cell populations. From the viewpoint of evolutionary biology, bet-hedging is traditionally viewed as an adaptation to environmental uncertainty (Simons, 2011), and phenotypic diversification enables species to survive environmental fluctuations. Here we argue that bet-hedging arises when complex cancers are generated through oncogenesis, despite the fact that this process is inefficient and wasteful. Although bet-hedging does not maximize expected fitness within a generation, it reduces fitness variance and hence maximizes fitness across generations under environmental unpredictability (Simons, 2011).

BET-HEDGING AS EVOLUTIONARY RESPONSE TO UNPREDICTABLE TUMOR ENVIRONMENT

In ecological settings, long-term unpredictable selection is expected to result in the evolution of bet-hedging strategies and development of either or both conservative (i.e., insurance policy) and diversifying (i.e., risk spreading) bet-hedging traits (Simons, 2011). Progenitor cancer cells in de novo tumors could employ either, but most likely both, adaptive strategies: (1) short-range

migration/dissemination of early metastatic cancer cells (that potentially lie dormant for decades) to provide a hedge against seasonal, but unpredictable onset of disastrous "predation" by the immune system (Eyles et al., 2010; Röcken, 2010), or (2) generation of an array of phenotypes (subclones of primary tumor cells) to facilitate cooperation and to reduce the risk of extinction (Simons, 2011). The evolution and appearance of late disseminating metastatic phenotypes may potentially be an adaptive bet-hedging response (both conservative and diversifying) to declining resources once primary tumors reach their growth limit and the carrying capacity of their microenvironment. Other evolutionary responses to environmental variance, such as adaptive tracking and adaptive phenotypic plasticity, are constrained by various factors: adaptive tracking is impeded under extreme environmental changes (e.g., switching between the hypoxic and oxygen-rich environments of primary tumors and the bloodstream), while adaptive phenotypic plasticity is only an ideal solution in a conceivable/predictable environment. Thus, development and survival under the broad array of circumstances that cancer cells experience in the lifetime of their host may be enhanced by bet-hedging strategies generating high ITH. It is important to mention that this perspective does not exclude the possibility that adaptive tracking and phenotypic plasticity also applies to cancer cells (but at later stages of tumor progression), but rather we propose that bet-hedging (and hence ITH) is the initial attribute of de novo cancer cells as adaptation to the organisms' unpredictable environment.

PREDICTIONS AND IMPLICATIONS

Even though bet-hedging could facilitate de novo development of tumor complexity, it is expected to be a slow process given that only a few useful components are produced per time unit. The formation of solid tumors by bet-hedging is thus expected to be a slow phenomenon. This is in accordance with solid tumors generally occurring late during the life, even if several other explanations are also fully valid (Merlo et al., 2006). In addition, in contrast with organisms that have practiced bet-hedging for millions of years, bet-hedging in our hypothesis is a de novo trait favored by oncogenic selection (i.e., because each cancer reinvents the wheel). This short evolutionary time may explain why bet-hedging here yields an abundance of nonfunctional/aberrant cells, instead of producing a variety of different but functional entities, as is usually observed when bet-hedging arises through natural selection on organisms. This explanation explains the variety of genomic aberrations among cancers of the same histological type, to the extent that no two tumors are thought to show an identical somatic genetic aberration profile (Lipinski et al., 2016). This model also predicts that in liquid environments like blood, where cell competition for resources and space limitations are relaxed, oncogenic selection should rather favor highly proliferative clones that do not cooperate. The futility of building cooperative tumor systems in this case may explain why cancer cells from liquid tumors do not aggregate and are on average less heterogeneous than in solid tumors (Alexandrov et al., 2013) (i.e., low selection for bet-hedging). Also because the dynamics of liquid tumors would not rely on a slow bet-hedging process, it could explain why they can reach, all things being equal, stages that are detrimental for health earlier in life (e.g., leukemia) compared to solid tumor cancers.

CONCLUDING REMARKS

Although the applications of an evolutionary perspective in human health research vary depending on the disease under study (Williams and Nesse, 1991), it is increasingly accepted that cancer is a process that follows Darwinian evolution (Aktipis and Nesse, 2013; Thomas et al., 2013). ITH is central in this reasoning since it provides the substrate from which somatic cellular selection and evolution can occur, leading to malignancy, with its many manifestations: neoangiogenesis, evasion of the immune system, metastasis, and resistance to therapies, and sometimes contagion (Ujvari et al., 2016). Recently, Ling et al. (2015) cast doubt on the selected nature of ITH, mainly because the great variation that can occur even in small tumors seems incompatible with predictions made by classical Darwinian reasoning. Here, we provide a conceptual framework proposing that ITH itself could also directly result from a selective process: bet-hedging. We cannot exclude in our hypothesis that there is an underlying ancestral bet-hedging program normally repressed that cancer cells are able to reactivate following mutations (Soto and Sonnenschein, 2011; Vincent, 2012) (see Chapter 16). We cannot exclude either that ultimate reasons other than the one proposed here (e.g., immune escape) also explain why tumorigenesis implies the concomitant selection of bet-hedging from the first malignant steps. Further work is necessary to determine whether or not classical evolutionary/ecological scenario (e.g., tumor heterogeneity would be the consequence of a heterogeneous landscape selecting for different phenotypes that are adaptive to these different microenvironments) can successfully explain the striking level of ITH observed in most tumors, or whether it is necessary to invoke bet-hedging processes. In the latter scenario, the true ultimate causation will remain to be determined.

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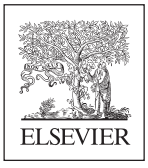
ECOLOGY AND EVOLUTION OF CANCER

Edited by

BEATA UJVARI
Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia

BENJAMIN ROCHE
UMMISCO (International Center for Mathematical and Computational Modeling of Complex Systems), UMI IRD/UPMC UMMISCO, Bondy, France; MIVEGEC (Infectious Diseases and Vectors: Ecology, Genetics, Evolution and Control), UMR IRD/CNRS/UM 5290, Montpellier, France; CREEC (Centre for Ecological and Evolutionary Research on Cancer), Montpellier, France

FRÉDÉRIC THOMAS
MIVEGEC (Infectious Diseases and Vectors: Ecology, Genetics, Evolution and Control), UMR IRD/CNRS/UM 5290, Montpellier, France; CREEC (Centre for Ecological and Evolutionary Research on Cancer), Montpellier, France



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