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Abstract

Stochastic gene expression plays a leading developmental role through its contribution to cell differentiation. It is also proposed to promote phenotypic diversification in malignant cells. However, it remains unclear if these two forms of cellular bet-hedging are identical or rather display distinct features. Here we argue that bet-hedging phenomena in cancer cells are more similar to those occurring in unicellular organisms than to those of normal metazoan cells. We further propose that the atavistic bet-hedging strategies in cancer originate from a hijacking of the normal developmental bet-hedging of metazoans. Finally, we discuss the constraints that may shape the atavistic bet-hedging strategies of cancer cells.

KEYWORDS
atavism, bet-hedging, cell-cell heterogeneity, cellular plasticity, oncogenesis, stochastic gene expression, transcriptional diversity

INTRODUCTION

Random cell-to-cell phenotypic fluctuations originating from stochastic gene expression (the so-called gene expression noise) are now considered to be major contributors to the differentiation processes in multicellular organisms.[1–6] Indeed, high gene expression stochasticity, manifested as strong fluctuations in the abundance of expressed molecules at the single-cell level, and the subsequent highly variable gene expression patterns, are necessary for developmental "choices".[1] For example, genome-wide transcriptional variability from cell-to-cell precedes cell fate decisions in hematopoietic progenitor cells.[7] Also, single-cell transcriptional diversity, which is the number of expressed genes per cell and is globally correlated to the level of gene expression stochasticity (both phenomena occur thanks to a more permissive chromatin (Figure 1)), is a hallmark of developmental potential.[8] This explains the mixed-lineage states and patterns of gene expression revealed by single-cell analyses in progenitor cells before commitment, especially in the hematopoietic system.[9,10] Interestingly, lineage-specific transcription factors from alternate lineages are co-expressed at the protein level in early progenitor cells, as exemplified in human erythropoiesis,[11] and cell fate decisions occur through gradual rather than abrupt quantitative changes in factor abundance.

Differentiation is then linked to the progressive reduction in chromatin accessibility during lineage commitment,[12,13] and to a decrease in transcriptional diversity.[8] Therefore, it can now be proposed that the looser chromatin in less mature cells permits a more stochastic gene expression and a wider sampling of the transcriptome, whereas chromatin accessibility, transcriptional diversity and expression stochasticity are restricted in more differentiated cells as they specialize[1] (Figure 1). It is of note that an increase in cell-to-cell gene expression variability is observed in vitro as hematopoietic progenitor cells start differentiating when environmental constraints (medium composition) that maintained the progenitor state are released, whereas variability drops to levels far below the initial level as cells become terminally differentiated.[2,3] A similar phenomenon is observed in vivo in mouse embryonic development, in which an increase in transcriptional noise is observed prior to

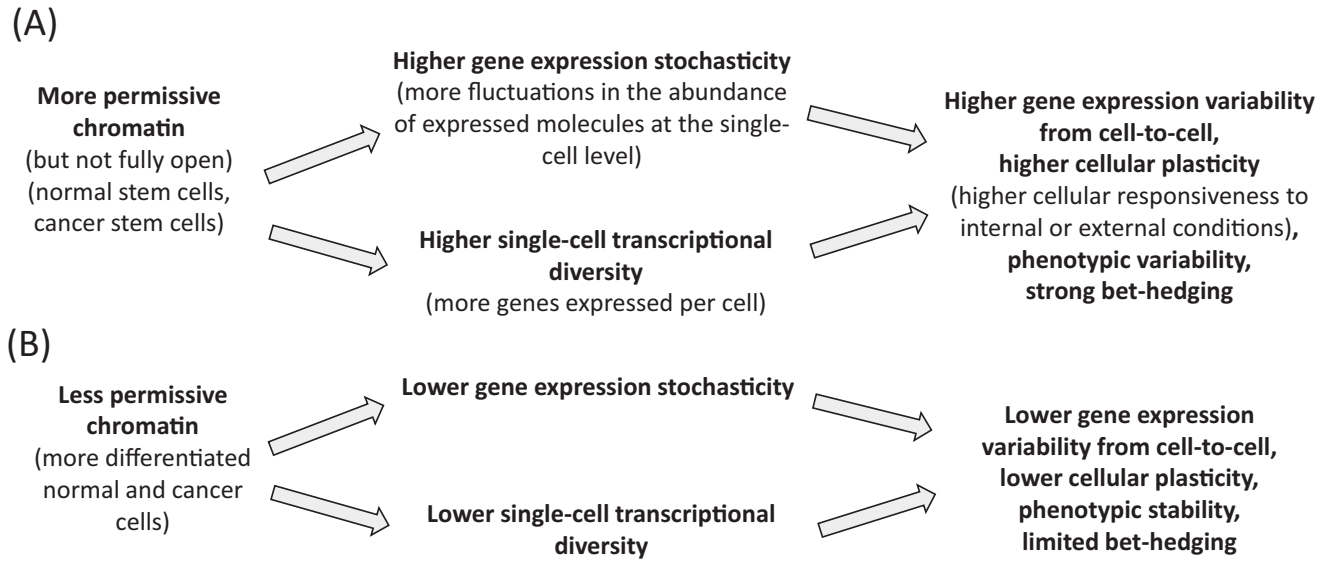

FIGURE 1 Main non-genetic differences between normal/cancer stem cells and more differentiated normal and cancer cells. (A) The more permissive chromatin in normal/cancer stem cells generates both a more pervasive expression of the genome, enhancing single-cell transcriptional diversity, and a higher gene expression stochasticity (more fluctuations in the abundance of expressed molecules at the single-cell level). These phenomena produce higher gene expression variability from cell-to-cell, higher cellular plasticity which corresponds to a higher cellular responsiveness to internal or external conditions, phenotypic variability, and strong bet-hedging. Nevertheless, this chromatin is not completely open (fully permissive) because if there is no stochasticity for genes that are always transcribed. Stochasticity requires chromatin that is neither perfectly closed, nor perfectly open. The chromatin should occasionally open up to generate stochasticity, so it should only be partially permissive. (B) In more differentiated normal and cancer cells, the chromatin is globally less permissive (more closed), reducing single-cell trancriptional diversity and gene expression stochasticity. This also reduces gene expression variability from cell-to-cell and cellular plasticity, and allows phenotypic stability and only limited bet-hedging

lineage commitment from implantation to early gastrulation, with possible consequences for symmetry breaking and cell fate decision-making.[4] A role for gene expression noise in early embryonic cell fate commitment was proposed 15 years ago[14] and is still widely discussed.[15]

There is still controversy over the role of gene expression variability in cell fate decision-making and the fact that the observed changes in variability can drive differentiation [16]. However, it is widely accepted that stochastic gene expression can generate random phenotypic fluctuations and cell states that likely induce differentiation. This can be seen as a "developmental bet-hedging strategy" that has similarities with the bet-hedging strategies observed in unicellular organisms,[17] where gene expression variability diversifies functional roles across a population of cells.[18,19] This is also coherent with the observed stochasticity in cell differentiation processes (for instance, see[5] for a detailed description of stem cell differentiation as a stochastic process and[6] for a review).

Here, we explore the differences between these two types of bet-hedging strategies; we then argue that phenotypic diversification in cancer cells could also rely on bet-hedging phenomena more similar to those observed in unicellular organisms than to those observed in developmental multicellular systems. We propose that cancer cells use "atavistic bet-hedging strategies," which constitutes a perversion of the way random single-cell phenotypic fluctuations are exploited in multicellular organisms.

DIFFERENCES BETWEEN DEVELOPMENTAL AND ATAVISTIC BET-HEDGING

The most cited noise-based model of differentiation relies on the notion of "attractor" in the dynamical-systems theory.[20] Within this theory, the global regulatory network (GRN) generates attractor states that are meta-stable and that can be regarded as distinct cell types (the stem cell state being the original attractor).[20] Noise-induced transition between attractors can occur when random expression fluctuations are sufficient to destabilize the current attractor state and generate a switch towards another attractor. Cell-to-cell communication can be considered a way to exploit and buffer intercellular variability,[15,21] but the attractors are fundamentally encoded in the GRN and allow the embryo and the differentiating tissue to self-organize.

In a more Darwinian view of differentiation, we and others have suggested that these varied patterns could act as a substrate for selection by environmental cues through a "chance-selection" process.[6,22,23] In this case, developmental bet-hedging processes would consist of randomly generated phenotypic fluctuations among which some gene expression patterns would later be stabilized and selected if the generated phenotypes were adequate in the current environment. Thus, this variability would reflect the need to switch to different fates based on specific selective external cues that stabilize specific chromatin structures.

Of note, this type of stabilization occurs outside of development and has been explored in multiple works for non-developmental bet-hedging,[24–26] so it is not specific to development. Nevertheless, in the developmental context, this would allow integration of the cells within the developing or renewing tissue through the establishment of direct or distant cell—cell interactions.[6,22] In that sense, developmental bet-hedging would be specific and different than other types of bet-hedging: not in its molecular origins but rather in its consequences in term of cellular integration in a stably functioning structure in which differentiation, equivalent to phenotypic stabilization produced by cell signaling from the established cell—cell interactions, and quiescence would be linked to the decreased level of gene expression noise and the progressive chromatin closure originating from environmental cues. Thus, a state with a chromatin structure more typical of a fully differentiated state would be much too resistant to switching, due to the stable cell-cell interaction network that has progressively taken place during differentiation and canalized cells towards differentiation.

We already mentioned that this progressive stochasticity-based cell differentiation and specialization is associated with formation of the multicellular organism in the developmental context, with the cells being ultimately controlled at the proliferation level and integrated into a cell community functioning for the benefit of the whole organism.[27] This canalization process leading to cell differentiation can be viewed as a constrained random process consisting of a permanent interplay between the stochastic dynamics of biochemical reactions at the cellular level and the environmental constraints that leads to a stabilized state of equilibrium.[6] Quiescence and integration into the functioning of the multicellular organism result from an evolutionary history in which a hereditary "memory" has been established based on the fact that these stochastic processes have been exploited and proved useful in the past to build multicellularity.

On the contrary, bet-hedging processes in unicellular organisms are used to specialize cells with the aim of maximizing cell proliferation, for example, by exploiting alternative carbon sources or adapting to fluctuating environments.[25,28,29] This microbial bet-hedging has been characterized and is different from developmental bet-hedging in that it does not aim to integrate cells into a cooperating community of quiescent cells working for the whole community (the multicellular organism), but is rather aimed at adaptation through specialization or re-specialization for the cell's own benefit. In this case, the subsequent decrease in cell stochasticity associated with specialization is not associated with quiescence, as it is for differentiated cells in multicellular organisms, but with maximized proliferation when environmental conditions are favorable[27] (if these conditions become too unfavorable, many bacteria and fungi can also enter a quiescent, or dormant, non-replicating state, e.g., persistence with/tolerance to antibiotics[30]). While it remains basically a selfish strategy to benefit the survival and proliferation of the individual cell, this strategy does not exclude the establishment of cooperation with subpopulations having different phenotypic behaviors or even with other organisms. For instance, an example of social behavior originating from phenotypic fluctuations is observed in the budding yeast *Saccharomyces cerevisiae*, linked to the *FLO11* locus. The adhesin Flo11 functions in cell—cell and cell—surface adhesion, and has been hypothesized to function in cell—cell recognition and allows for cell—cell homotypic interactions.[31] *FLO11* is heterogeneously expressed in clonal populations, which is likely to be adaptive, because this variegated *FLO11* expression leads to increased biomass and space usage in nutrient-limiting environments.[32] Indeed, stable proportions of $Flo11^{+}$ and $Flo11^{-}$ cells result from differential expression of *FLO11* in clonal *S. cerevisiae* biofilm colonies, and differentiated $Flo11^{+/-}$ colonies, composed of adhesive and non-adhesive cells, have a strong growth advantage over undifferentiated colonies.[32] Moreover, works on natural genetic variations in the *FLO11* locus found that particular *FLO11* alleles were associated with increased sociality, and strongly suggest selection on heterogeneous *FLO11* expression in clonal lineages.[33] Thus this example seems to provide a good illustration of the role of phenotypic fluctuations in giving rise to cooperative behavior between different phenotypes in microbial populations.

Interestingly, cancer cells seem to harbor similar trends to exploit cellular stochasticity through bet-hedging processes.[34,35] Even though the strategies of retaining high variability at the phenotypic level, and of being more phenotypically stable may both appear to be losing strategies in the cancerous environment, the combination and fluctuation between them could constitute a Parrondo's paradox by being a winning strategy resulting from two losing strategies.[35] In other words, transitions between a state with strongly fluctuating expressions and lack of differentiated phenotypes that can be called cancer stem cell (CSC) in analogy with the properties of the "normal" stem cells,[36] and more differentiated states, would be advantageous in an evolutionary perspective.

In line with this hypothesis, we propose that bet-hedging processes, which can be called "atavistic bet-hedging", are crucial in cancer cell populations because they could provide cells with new gene expression patterns that could allow local adaptation to a given environment. This strategy would be associated with decreased stochasticity and improved cell proliferation, but with the possibility that cell stochasticity (and plasticity which is the cellular responsiveness to internal or external conditions and can result in higher phenotypic variability) could reincrease and restore atavistic bet-hedging if cells are no longer on an adaptive summit. This is closer to a unicellular bet-hedging strategy than a developmental bet-hedging strategy.

Thus, at any moment of cancer development, a mix of cells with new phenotypic combinations attained through atavistic bet-hedging strategies along with more specialized and proliferative cells would coexist. The spatial repartition of these subpopulations would depend on the tumor's structure, with variably fluctuating and challenging environments that would require variably adaptive capacities. For instance, at the surface of a solid tumor, where cells are confronted with the need to deeply modify well-structured tissue (even if it is already perturbed by paracrine molecules), bet-hedging is probably more important than in more internal zones of the tumor, where most of the cancer cells have reached a more stable state adapted to the cancerous microenvironment and where structuration is optimal for proliferation. In blood cancers, these same atavistic bet-hedging strategies could occur but may be less intensive because tissue barriers are less stringent. However, increased cell plasticity—even though it is considered the

result of an "oncogene-induced reprogramming" and not produced by a microenvironmental disruption—is now considered a key factor in leukemic cell function.[37] The early acquisition of cell plasticity as a consequence of an increase gene expression stochasticity can easily be the best way to exploit an atavistic, stochasticity-based bet-hedging process for cancer development.

To reach tumor structuration from cells adopting atavistic bet-hedging strategies, the establishment and maintenance of cooperative mechanisms are required. As is the case for developmental processes—but without the reproducible chance-selection processes based on developmental bet-hedging gained and optimized for ontogenesis across evolution—random single-cell phenotypic fluctuations would inevitably lead to cooperative behaviors, as observed in microbial communities.[29] This can be considered the basis for the structuration of tumors—as well as for multicellular organisms—but through an always-new process that has no heritable "memory" of previous stochastically based cancer development (except in the case of transmissible cancers; see below).

Finally, the ability to switch from higher or lower cellular stochasticity and its exploitation for maximizing cell proliferation through atavistic bet-hedging requires that cells are no longer canalized nor maintained under the control of a healthy microenvironment. We next consider how cancer cells transit from developmental to atavistic bet-hedging.

FROM DEVELOPMENTAL TO ATAVISTIC BET-HEDGING IN CANCER CELLS

Many studies have noted numerous cancer driver mutations in histologically normal human tissues,[38–44] supporting investigations that have recently reconsidered the role of host microenvironment perturbation in tumor development.[45–48] These studies highlighted the fact that mutations alone are not sufficient for tumor development, and that tissue disruption has a more active role than previously thought in the early steps of oncogenesis. However, the obligatory preexistence of genetic alterations in healthy tissue that develops cancer is rarely questioned. Only a few studies,[49,50] including ours,[51–53] have proposed microenvironmental changes and the disruption of multicellular organization as possible initiating events in oncogenesis.

In particular, we suggested that a link between the disrupted microenvironment (perturbation of the environmental constraints acting at the tissue level through cell—cell interactions and communications, and at the organism level through endocrine, immunity, and blood networks) and enhanced cellular stochasticity was responsible for the loss of stable and well-defined phenotypic and differentiated features and/or the loss of canalization of normal stem cells.[51–53] This microenvironmentally-induced enhanced stochasticity would not only produce cells with stem cell-like properties (called CSC), but also produce a switch from developmental to atavistic bet-hedging in cancer cells. The role of phenotypic plasticity and bet-hedging processes have already been highlighted in the context drug resistance and tumor relapse in prostate cancer by giving rise to drug-tolerant persisters,[54] but we suggest here to adopt a broader perspective and to consider bet-hedging processes as a central phenomenon in tumor growth.

Indeed, as soon as the dynamically evolving environmental constraints no longer exert their canalizing effect, and as soon as the selective developmental process mentioned above can no longer occur because direct and/or indirect cellular interactions are disrupted, the random generation of single-cell phenotypic fluctuations can only serve as a substrate for bet-hedging strategies similar to those observed in microorganisms.

While the normal developmental process is also based on a principle of "chance-selection" where noise plays a major role, in the language of bifurcation theory, bifurcations have been "optimized" so as to be rapid, unique and unidirectional in response to the canalizing environment. On the contrary, in the disrupted cancerous environment, cells are freer to explore the phenotypic space and behave more stochastically in terms a mixed phenotypes, phenotypic reversibility, more in a unicellular mode.

This perspective certainly presents many convergences with the Davies and Lineweaver's 2011 atavism theory of cancer where the authors argued that cancer is due to an accidental reactivation of a highly conserved survival program encrypted in every eukaryotic cell and a reversion to "Metazoa 1.0".[55] However, in the present article and a previous one,[27] we rather consider a renewed atavism theory of cancer based on cellular stochasticity. Indeed, without the need to invoke an ancestral legacy, tumor adaptations may also result from a somatic evolution with selection of malignant cells that adopt a stochasticity-based microbial cell lifestyle which could be the most adapted given the new environmental constraints.[27]

Of note, this microenvironmental disruption would make the cancer cell phenotypes much more plastic with reversible transitions from more differentiated to more stem-like cancer cells during tumor growth, compared to the essentially irreversible nature of differentiation during orchestrated development. Even if this reversibility could also rely on broken regulatory links arising from mutations that can act as tumor "promotors",[51] it would mainly originate from to the disrupted microenvironment, making mutations not absolutely necessary, although unavoidable in tumor growth.

Therefore, a non-genetic stochasticity-based construction of the tumor architecture would then be possible and take place. Spatial organization and cooperative behaviors between distinct subpopulations would result from a selection process based on random phenotypic fluctuations produced by uncanalized enhanced stochastic gene expression rather than being the result of ontogenesis as for developmenting tissues, but following new dynamics because tumor development cannot be influenced by future environmental conditions.

RELATIONSHIPS WITH THE PHENOTYPIC COMPOSITION OF THE TUMORAL GROUP

It is increasingly argued that tumor growth does not solely rely on the quantitative rate of cell proliferation inside the tumor, but also on the way the produced cells succeed in forming a functional group.

Tumors consist of a consortium of cooperating malignant clones organized into functional compartments, with a division of labor among these compartments.[56–58] The resulting tumoral group phenotypic composition (GPC) is therefore key to understanding the ecological and evolutionary trajectories of malignancies.[59] However, because malignant cell proliferations rely on atavistic bet-hedging, it is expected that they will produce different possibilities of tumoral GPCs (e.g., depending on which cells have a selective advantage inside the tumor at a given time). Since evolution is not affected by the future and because there is no encoded program to produce optimal tumor tumorigenesis, atavistic bet-hedging is expected to regularly yield non-optimal, or even detrimental, tumoral GPCs.[60] This lack of adequacy between optimal and observed GPCs can in theory stop or slow down tumor progression. In addition, there is not only one optimal GPC that would provide the tumor with adequate combinations of different and complementary cells during tumorigenesis. For instance, tumors of different volumes or in different organs have different needs, and they also have different interactions with their microenvironment. Producing tumoral GPCs via atavistic bet-hedging that adequately vary with tumor stage and the microenvironment is improbable, and this explains why only a tiny proportion of emerging neoplasia succeed at each step and evolve into metastatic cancers. The inability of the atavistic bet-hedging to generate tumors with adequate GPCs at each step would also explain why we accumulate various stages of stable neoplasms in the body through time that can sometimes even regress.[61]

A major constraint limiting the possibility that malignant atavistic bet-hedging can evolve over evolutionary time to produce progressively enhanced GPCs is that cancers are usually not transmissible. Therefore, there is no possibility of selecting bet-hedging options that would progressively yield tumors with optimal levels of functionality. In comparison, a complex organism with different organs is also characterized by its GPC; however, since individuals reproduce, there is the possibility of selecting GPC combinations that are optimal for organismal development, functioning, and reproduction in its environment.

THE CASE OF TRANSMISSIBLE CANCERS

In contrast to most cancers that die with their host, transmissible malignancies are composed of clonal cell lineages that have acquired the ability to pass from host to host.[62–66] These cancers truly evolve as novel parasitic life forms and are not reinvented each time they emerge as classical malignant cells.[60,65] Because of this important particularity, we might expect that these transmissible cancers undergo evolution and/or reversion (malignant cells derive from healthy cells), from an atavistic bet-hedging to a more canalized developmental one, allowing optimization of the adaptive traits related to the parasitic lifestyle. To our knowledge, this question has not yet been addressed. Interestingly, the age of transmissible cell lines is highly variable between the few biological models that have been identified, ranging from a few years (e.g., 26 for devil facial tumor disease in Tasmanian devils) to about 10 000 years (for canine transmissible venereal tumor). We cannot exclude that there would be intermediate forms of transmissible cancers ranging between atavistic bet-hedging and developmental one depending on their age. Ultimately, a more reproducible GPC may occur in the transmissible ones, albeit going in the direction of proliferation, and not for the control of proliferation for the benefit of the whole organism. Studies mimicking the transmission of malignant cells in an experimental evolutionary context (e.g.,[67]) could help explore this hypothesis. Also, given that we currently underestimate the number of transmissible cancers,[68] it seems likely that the future discovery of novel models will help to address this question.

CONCLUDING REMARKS

Since the late Precambrian, multicellularity evolved independently on multiple occasions, but each time it required that the newly evolved multicellular phenotypes had the ability to control their cellular proliferation.[69,70] The achievement of this fundamental condition was not incompatible with the presence of high levels of stochasticity in gene expression, and it provided mechanisms that could subsequently constrain gene expression patterns to a certain homogenization during differentiation. As a form of collateral effect, it is increasingly argued that tumorigenesis could be initiated by disrupting constraints that canalize cells towards their stable phenotypic state, yielding a form of cellular speciation relying on increased cellular stochasticity.[27] Whether this phenomenon is a reversion to prior phylogenetical capabilities or conversely corresponds to newly acquired cellular traits remains an ongoing debate. In any case, increased cellular stochasticity does not mean that there are no longer constraints on this trait. Inappropriate levels of stochasticity can yield poorly functional GPC within a tumor, even if this phenomenon remains in itself unlikely to trigger selective processes favoring more adapted adjustments (except with transmissible cancers). Another constraint—in this case able to shape the level of stochasticity—is related to the degree of environmental adversity for cancer cells in different organs of a multicellular organism's body. Giraudeau et al.[71] argued that for ecological and evolutionary reasons, important variability exists between organs in their capacity to suppress tumors. When a tumor develops in an organ exerting strong selection pressure on malignancies, cancer cells (at least those that survive and are detectable) are expected to display a large range of survival strategies, like hyper-variable phenotypes relying on bet-hedging to persist, or conversely variants with low variabilities that succeed in escaping anticancer defenses. Because these constraints are not observed in organs that are less able to eradicate tumors, selective pressures favoring extreme survival strategies are relaxed, and this is likely to allow a wider range of cellular stochasticity levels. Therefore, even if bet-hedging strategies in cancer cells are similar to those observed in unicellular organisms, it is still important to consider the local specificity of the multicellular body viewed as a habitat for cancer cells.

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AUTHOR CONTRIBUTIONS

Jean-Pascal Capp and Frédéric Thomas formulated the hypotheses and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable as this is a perspective article.

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