Cancer: More than a geneticist’s Pandora’s box

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Despite identical genetic constitution, a cancer cell population can exhibit phenotypic variations termed as non-genetic/non-mutational heterogeneity. Such heterogeneity – a ubiquitous nature of biological systems – has been implicated in metastasis, therapy resistance and tumour relapse. Here, we review the evidence for existence, sources and implications of non-genetic heterogeneity in multiple cancer types. Stochasticity/noise in transcription, protein conformation and/or external microenvironment can underlie such heterogeneity. Moreover, the existence of multiple possible cell states (phenotypes) as a consequence of the emergent dynamics of gene regulatory networks may enable reversible cell-state transitions (phenotypic plasticity) that can facilitate adaptive drug resistance and higher metastatic fitness. Finally, we highlight how computational and mathematical models can drive a better understanding of non-genetic heterogeneity and how a systems-level approach integrating mathematical modeling and in vitro/in vivo experiments can map the diverse phenotypic repertoire and identify therapeutic vulnerabilities of an otherwise clonal cell population.

Keywords. Biological noise; drug-tolerant persisters; epithelial–mesenchymal plasticity; multistability; non-genetic heterogeneity; PAGE4; phenotypic plasticity

1. Introduction

Cancer is thought to originate from an individual normal cell that gains genetic mutation(s) offering it growth advantage over other cells. A clonal population of cells can arise from this ‘first’ cancer cell; this population has identical genetic composition (Nowell 1976; Greaves and Maley 2012). As time progresses, some of these cells can gain additional mutations, thus leading to sub-clones, some of which can be more fit as compared to others and thus undergo natural selection to become the predominant sub-clone(s). Recent studies across clonal populations in cancer cells as well as other biological contexts (such as microorganisms; Davidson and Surette 2008) have proposed that phenotypic variations exist among genetically identical cells (Brock et al. 2009). These phenotypic variations are referred to as non-genetic heterogeneity (NGH), highlighting their non-genetic/non-mutational origin. Such non-genetic mechanisms can include a combination of various processes such as stochasticity or noise in gene expression (Balážsi et al. 2011), asymmetry in distribution of molecules during cell division (Huh and Paulsson 2011), variability in epigenetic status of cells (Bell and Gilan 2020), promiscuity in protein–protein interaction networks due to disorder in protein structures (Lin et al. 2019) and ability of cells to exhibit multiple phenotypes (multistability) as a result of feedback loops embedded in a gene regulatory network (Evans and Zhang 2020; Hari et al. 2020).
Consequently, cells in a clonal population can have variability in their protein levels, chromatin accessibility status, metabolites, etc., which can eventually manifest as different phenotypes. Non-genetic heterogeneity has been shown to offer survival advantages to cells in fluctuating environments such as drug treatment, hypoxia and nutrient deprivation, by facilitating ‘bet-hedging’, an evolutionary strategy to achieve fitness across environmental conditions (Veening et al. 2008a, b; Pisco and Huang 2015; van Boxtel et al. 2017; Bell and Gilan 2020; Sahoo et al. 2021a). However, unlike genetic heterogeneity that has been extensively investigated for decades (McGranahan and Swanton 2017), understanding the underlying mechanisms and implications of non-genetic heterogeneity in cancer research is still in its infancy (Marine et al. 2020; Barzgar Barough 2021; Lewis and Kats 2021; Shlyakhtina et al. 2021).

The ‘one gene–one enzyme’ hypothesis postulated in 1941 (Beadle and Tatum 1941) was among the first to propose a one-to-one mapping between the genotype and phenotype. However, as we now realize, a phenotype is the outcome of extensive cross-talk among many biological factors that form regulatory networks at various length scales and time scales, revealing pleiotropy (Tyler et al. 2009) in the genotype–phenotype map. Regulatory networks in biological systems consist of multiple feedback loops which can produce more than one phenotype depending upon cell-intrinsic (e.g., rates of transcription, translation and protein degradation) and cell-extrinsic (e.g., temperature, oxygen and nutrient availability) factors (Brandman and Meyer 2008). Thus, despite having identical genetic composition, clonal cancer cells can display differences in phenotypic properties such as growth rates (Vega et al. 2004), tumour initiation capabilities (Mani et al. 2008; Pasani et al. 2021) and the ability to evade therapeutic attacks (Sharma et al. 2010; Paek et al. 2016; Shaffer et al. 2017). Intriguingly, cells can switch back and forth among these different phenotypes, driving reversible changes which may or may not be inherited, unlike mutational effects which are irreversible in nature and ‘hard-wired’ to be passed on to progeny.

In this review, we first discuss the tacitly assumed essentiality and sufficiency of mutations for some aspects of cancer progression and highlight some observations that cannot be completely explained by the somatic mutation theory alone. Next, we provide evidence of non-genetic heterogeneity observed in clonal cancer cell populations and offer mechanistic details for some of its sources. Finally, we discuss the implications of such heterogeneity in various hallmarks of cancer. We also highlight the contribution made by mathematical models to understand non-genetic heterogeneity and suggest that a systems-level understanding integrating theory and experimental observations may provide a better understanding of non-genetic heterogeneity and how this knowledge may be leveraged to restrict aggressive clinical outcomes.

2. Are mutations necessary for cancer progression?

For many years, DNA mutations have been considered to be the main cause of cancer initiation wherein a mutation in an oncogene or tumour suppressor gene can drive abnormal cell growth. Such mutations are implicitly assumed to be necessary and sufficient for cancer progression, leading to the most popular and widely accepted concept in the field of cancer biology – somatic mutation theory (SMT) – which posits a mutation in a single somatic cell as the first step of cancer (Sonnenenschein et al. 2014). This mutation in a cell is considered to be sufficient to perturb its cell cycle regulation, leading to uncontrolled cell proliferation. However, many observations in cancer biology do not appear, at least prima facie, in consensus with SMT, such as spontaneous regression of childhood neuroblastoma without any cytotoxic therapy (Haas et al. 1988), ependymomas in children (MacK et al. 2014), reprogramming of cancerous cells to normal cells when implanted into normal microenvironments (Mintz and Illmensee 1975; McCullough et al. 1997; Kasemeier-Kulesa et al. 2008; Bussard et al. 2010), stromal induction of carcinogenesis in epithelial cells (Barcellos-Hoff and Ravani 2000; Maffini et al. 2004, 2005; Barclay et al. 2005), and the cycling of hepatic cells from cancerous to normal (dormancy) by the dialling up or down of the wild-type MYC oncogene (Shachaf et al. 2004; Shachaf and Felsher 2005).

Thus, an alternative to SMT, although yet not that popular, after SMT has been proposed recently – tissue organization field theory (TOFT) (Soto and Sonnenschein 2011). TOFT considers cancer to be a tissue-based disease (instead of a cell-based disease as considered by SMT) akin to development gone awry. According to TOFT, cancer arises as a result of simultaneous occurrence of two steps: a disturbed interaction between parenchyma and stroma resulting in altered tissue organization and a weaker inhibitory control exerted by tissues over cell proliferation (Sonnenenschein and Soto 2015). TOFT is further supported...
by observations that primary tumours can metastasize to only few specific organs, indicating that the stroma of an organ plays an important role in determining whether or not cancer cells attached to it can start metastatic growth in it (Tarin 2011). This concept is endorsed by recent observations suggesting that cancer cells can carry their own stroma (‘soil’ as per Paget’s ‘seed and soil’ hypothesis; Paget 1889) for successful metastasis (Duda et al. 2010). While the discussion about similarities, differences and complementarity between SMT and TOFT is beyond the scope of this review, the dynamics of cancer metastasis offers an intriguing scenario to dissect the role of mutations in cancer progression.

Despite tremendous advances in high-throughput single-cell sequencing in the last decade, no unique mutational signature has yet been identified for metastasis (Celià-Terrassa and Kang 2016; Welch and Hurst 2019). However, non-genetic variability due to network architecture between metastasis suppressors and metastasis drivers has been shown to generate a subpopulation of pro-metastatic cells without gaining any additional genetic changes (Lee et al. 2014). This variability further leads to cellular or phenotypic plasticity – the ability of cells to reversibly switch their phenotypes, and has been proposed as a hallmark of metastasis (Bhatia et al. 2020; Biswas and De 2021; Sacchetti et al. 2021). One of the key axes of phenotypic plasticity during metastasis is epithelial–mesenchymal plasticity (EMP), where cells can transition among a range of phenotypes spread over a spectrum ranging from more epithelial (usually more adhesive and less invasive) to more mesenchymal (usually more invasive and having reduced cell–cell adhesion) (Gupta et al. 2019; Pastushenko and Blanpain 2019). EMP can have transcriptional (Yang et al. 2004; Cieply et al. 2012; Ocaña et al. 2012; Roca et al. 2013; Subbalakshmi et al. 2020) and/or epigenetic (Ruscetti et al. 2016; Jia et al. 2019; Nihan et al. 2019; Serresi et al. 2021) regulatory control. Thus, the dynamics of complex interconnected networks at various levels of regulation can dictate the propensity of a cell to slide along the ‘EMP axis’. Moreover, EMP can influence other axes of plasticity such as stemness/tumour initiation potential (Celià-Terrassa et al. 2012; Jolly et al. 2015), resistance to cell death caused by anchorage independence (anoikis) (Huang et al. 2013), resistance to various targeted therapies and immunotherapy across cancers (Chouaib et al. 2014; Tripathi et al. 2016; Dongre et al. 2017; Sahoo et al. 2021a; Shafran et al. 2021), increasing cancer cell fitness during the metastatic cascade. Thus, EMP is a canonical example of phenotypic plasticity and consequent non-genetic heterogeneity implicated in successful metastasis.

Metastasis is a highly inefficient process (Luzzi et al. 1998; Cameron et al. 2000) during which the local microenvironment of disseminated cells is quite dynamic, and cells need to adapt rapidly to survive the bottlenecks they face. The timescale of obtaining the ‘right’ mutation that can tunnel cells through that bottleneck is over multiple cell divisions, thus being largely inconsequential to the probability of cells surviving that bottleneck. Moreover, while a newly acquired mutation may enhance the survival likelihood of a circulating tumour cell for a given bottleneck, it can compromise the ability of the cell to adapt to additional bottlenecks due to irreversible changes in its phenotypic repertoire. Put together, fast and reversible adaptations at a phenotypic level (i.e., Shachaf and Felsher 2005, non-genetic) seem to be playing an instrumental role in enabling cancer metastasis as compared to slow and irreversible adaptations available at a genetic level. Hence, it is not surprising that while cells from various sub-clones of the primary tumour have been seen in circulating tumour cells and capable of metastasizing (Lyberopoulou et al. 2015; Simeonov et al. 2021), no unique mutational footprints have yet been deciphered, unlike other hallmarks of cancer, for which mutations in various oncogenes and/or tumour suppressor genes have been pinpointed (Hanahan and Weinberg 2011; Mantovani et al. 2019). Such plasticity during metastasis can lead to phenotypic (non-genetic) heterogeneity, as witnessed in circulating tumour cells (CTCs) across cancer types (Yu et al. 2013; Bocci et al. 2021). Consistent with the observed impact of plasticity on the evolvability of cellular traits (Fierst 2011), non-genetic heterogeneity has been identified to impact evolutionary dynamics in lung cancer beyond genetic heterogeneity as well (Sharma et al. 2019).

Besides metastasis, phenotypic plasticity and non-mutational heterogeneity have been implicated in the emergence of adaptive drug resistance (Boumahdi and de Sauvage 2020; Qin et al. 2020; Oren et al. 2021), particularly through drug-tolerant persister (DTPs) – a subpopulation of cells that can survive sustained therapeutic attack by entering a reversible slow-proliferation state (figure 1). DTPs adapt to environmental fluctuations through epigenomic, transcriptional and metabolic reprogramming events, and are capable of expanding into a colony (Shen et al. 2020b). Initially reported in lung cancer (Sharma et al. 2010), persisters have been reported in other cancer types as well such as melanoma and colorectal cancer (Hangauer et al. 2017; Shen et al. 2020a; Karki et al. 2021; Mikubo et al.
Interestingly, DTPs can act as a reservoir subpopulation through which genetically mutated cells can emerge to stabilize diverse drug resistance mechanisms at a longer timescale (Ramirez et al. 2016). Thus, genetic and non-genetic mechanisms can be thought to cooperate to allow cancer cell adaptation at different timescales during the emergence of drug ‘resistance’ (Salgia and Kulkarni 2018; Hayford et al. 2021).

3. Are mutations sufficient for cancer progression?

Investigations into mechanisms of cancer initiation have also questioned the sufficiency of genetic mutations in cancer cells. For example, transformation of a normal cell to a cancerous melanoma cell has been shown to be triggered by imbalance in physiological factors along with exposure to the environmental carcinogen ultraviolet B (UVB) (Berking et al. 2004). Using human skin grafting experiments in immune-compromised mice, it was found that exposing normal melanocytes to increased levels of fibroblast growth factor, stem cell factor and endothelin-3, along with exposure to UVB, could transform normal melanocytes to a cancerous melanoma within four weeks of treatment, while treatment with individual growth factor along with UVB had no effect (Berking et al. 2004). This study suggests that only an external carcinogen is not always sufficient to initiate cancer; instead, some internal physiological imbalance is crucial as a permissive key to trigger neoplastic transformation. Consistently, another study in UV-induced melanoma argues that the susceptibility or resistance of mice to develop cancer strongly depends upon the presence of variants in the modifier genes along with the pathogenic genetic mutation (Ferguson et al. 2019). Thus, apart from genetic mutation(s), the overall genetic make-up of an organism and/or perturbation in the local environment may contribute to the induction of carcinogenesis, offering possible reconciliation between SMT and TOFT.

A commonly asked question in cancer biology is, ‘If mutations in cancer-associated genes are sufficient for neoplastic transformation, why do normal cells carrying similar somatic mutations not get transformed and develop cancer?’ With advancements in DNA-sequencing technologies, it is now possible to detect low-frequency somatic mutations in normal cells (Kennedy et al. 2019). The existence of somatic mosaicism (genetically distinct somatic cells harboured by an individual through DNA structural abnormalities, epigenetic changes and errors in chromosome partitioning) is well established (Youssoufian and Pyeritz 2002). Thus, genetic instability is not necessarily a unique property of cancer cells but inherent to all somatic cells, further emphasizing the role of altered microenvironments and/or other permissive cues in tumour initiation (Lichtenstein 2018). For example,
deleterious, age-associated, somatic mutations in the TP53 gene, commonly associated with serous ovarian cancer, are also detected at a very low frequency in the peritoneal fluid from women without cancer (Krimmell et al. 2016). Similarly, cancer-associated somatic mutations arising due to clonal expansion of hematopoietic cells have been reported in healthy individuals (Genovese et al. 2014; Xie et al. 2014). Such mutations are also detected in solid tissues of healthy individuals such as skin, colon, endometrium, brain, etc. (Kennedy et al. 2019). Conversely, in ependymomas – common childhood brain tumours – no significant recurrent mutations were detected in a cohort (MacK et al. 2014).

Together, these studies provide a strong indication that the presence of genetic mutations may not always result in tumour initiation, and that other permissive cues within a cell and/or its local microenvironment may drive neoplastic transformation of normal cells and tumour progression as well.

4. Evidence of non-genetic heterogeneity in clonal population of cells

The evidence of non-genetic heterogeneity in clonal populations was first noted in microorganisms. For example, Dictyostelium discoideum shows a bimodal (two-peak) distribution of motility speed and calcium content within 15 minutes upon starvation. The observed differences also reverted within 15 minutes after restoration of the nutrient medium, indicating reversible state transitions (Goury-Sistla et al. 2012). Similarly, variations in the levels of intracellular cyclic AMP (cAMP) in Saccharomyces cerevisiae has been shown to be associated with heterogeneity in growth rate and in acute stress tolerance. Perturbing this heterogeneity can impact these functional traits: while increase in intracellular cAMP levels increases susceptibility to acute heat stress, PKA inhibition decreases it, suggesting that underlying population structures get altered in opposite directions by these perturbations (Li et al. 2018).

While cell-extrinsic perturbations can reveal non-genetic heterogeneity, it may also arise due to cell-intrinsic variations in functioning of homeostasis in cell organelles. For example, variability in mitochondrial membrane potential among cells was correlated with that in proliferation rate, stress tolerance and resistance to therapy in yeast, identified using high-throughput automated microscopy (Dhar et al. 2019). Similar to unicellular organisms (Elowitz et al. 2002), eukaryotes also exhibit non-genetic cell-to-cell variability. For instance, the clonal population of mouse hematopoietic cells showed a distribution of levels of the stem cell marker Sca-1. While the Sca-1 high subpopulation with increased expression of PU.1 preferentially differentiated to a myeloid lineage, the Sca-1 low subpopulation expressing higher levels of GATA1 showed greater preference towards erythroid differentiation, highlighting how stochasticity can impact lineage choice in mammalian progenitor cells (Chang et al. 2008).

More recently, a population of cancer cells with identical genetic background has been observed to show differential gene expression and protein levels and consequently functional readouts such as response to drugs and tumour initiation. Advancement in flow cytometry methods, single-cell transcriptomics, lineage tracing and fate-mapping techniques have provided increasing evidence of phenotypic heterogeneity in a genetically homogenous cancer cell population (Sasagawa et al. 2013; Celii-Terrassa et al. 2018; Karacosta et al. 2019; Specht et al. 2019; Cook and Vanderhyden 2020). Single-cell expression variability is not unique to cancer cells; it has been witnessed among homogenous population of non-cancerous cells too, with important functional implications. For instance, highly variable genes in lung airway epithelial cells were enriched with collagen formation; those in dermal fibroblasts were found to be involved with keratinization, and those in lymphoblastoid cells were enriched with cytokine signaling (Osorio et al. 2020).

Multilineage differentiation programs operated in solid tissues have been proposed as potentially responsible for non-genetic heterogeneity observed in cancer cells. For example, six molecular subtypes of normal Fallopian tube epithelium (FTE; cells of origin of serous ovarian cancer (SOC)) were identified using transcriptomic analysis of 4000 normal FTEs, which was used to deconvolute non-genetic heterogeneity observed in high-grade SOC (Hu et al. 2020). Similarly, using single-cell PCR gene-expression profiling, non-genetic transcriptional variability observed in human colon cancer was demonstrated to be similar to different lineages of normal colon epithelium (Dalerba et al. 2011). Different single-cell transcriptomics or proteomics methods are offering unprecedented insights into elucidating patterns of heterogeneity in a homogenous cell population. For example, co-sequencing of microRNA-mRNA in individual cells using the half-cell genomics approach showed that variability of microRNA levels may drive non-genetic heterogeneity among cells (Wang et al. 2019). Similarly, two distinct cell populations within a melanoma
tumour were observed to be characterized by variable expression levels of microphthalmia-associated transcription factor (MITF) (Tirosh et al. 2016; Rebecca and Herlyn 2020). Further, subpopulations with varying differential EphA cluster morphologies and intrinsic migration potential were observed in breast cancer cells using single-cell assays (Ravasio et al. 2020). Identification of such heterogeneity has revealed that multiple cancer subtypes may coexist within an individual tumour (Yeo and Guan 2017). Relative proportions of cells exhibiting distinct subtypes constituting a tumour are expected to be highly dynamic and under constant drug-induced evolutionary pressures.

An outstanding example of phenotypic plasticity in cancer is EMP, which includes transition of cells among epithelial (E), mesenchymal (M) and hybrid E/M states (Celià-Terrassa and Jolly 2020). Epithelial–mesenchymal transition (EMT) and its reverse mesenchymal–epithelial transition (MET) – which together constitute EMP – are fundamental processes in development and wound-healing where they facilitate the movement of cells from one location to another (Nieto et al. 2016). This property of EMT–MET is exploited by and benefits cancer cells, where it not only confers cell motility (Pearson 2019) but also is implicated in metabolic reprograming (Krebs et al. 2017; Jia et al. 2021), tumour-initiation potential (Grosse-Wilde et al. 2015; Kröger et al. 2019), multi-drug resistance (Shibue and Weinberg 2017), immune evasion (Chen et al. 2014; Dongre et al. 2017; Sahoo et al. 2021b) and eventually patient survival (Tan et al. 2014; George et al. 2017). Clonal population of cancer cells may display a dynamic EMP status depending upon the relative levels of inducers and/or stabilizers of mesenchymal states (EMT-inducers such as ZEB/SNAIL family members) and those of epithelial ones (MET-inducers such as GRHL, OVOL and miR-200 family members) which often regulate the cellular levels of one another through reciprocal feedback loops (Bralbetz and Bralbetz 2010; Kvakackova et al. 2021). Various stabilizers of hybrid E/M phenotypes such as NRF2 and NUMB can influence the cell-state transition rates among different phenotypes along the ‘EMP axis’ (Hong et al. 2015; Bocci et al. 2017, 2019b; Biswas et al. 2019).

At a cell morphological level, EGF-induced EMT in breast cancer cells can be classified into three distinct reversible morphological states and function in a dose-dependent manner: cobble, spindle and circular (Devaraj and Bose 2019). Similarly, using a Z-cad dual-sensor system with an epithelial and a mesenchymal marker together, dynamic changes in breast cancer cells undergoing EMT or MET can be observed, which can help isolate the subpopulation displaying mesenchymal properties from a population consisting of predominantly epithelial-like cells (Toneff et al. 2016). Moreover, the percentage of cells in E, hybrid E/M and M states at various timepoints during EMT induction can be quantified using such a sensor and/or single-cell RNAseq data, highlighting patterns of heterogeneity (Jia et al. 2019; Cook and Vanderhyden 2020; Deshmukh et al. 2021). Reversible changes in the frequency of epithelial and mesenchymal cell states have been seen not only in vitro but also in the circulating tumour cell (CTC) composition of cancer patients with each cycle of response to therapy (Yu et al. 2013). Further, there may be heterogeneity in hybrid E/M states as well, as identified in primary skin and mammary tumours (Pastushenko et al. 2018) as well as in breast and lung cancer cells (Hong et al. 2015; Schliekelman et al. 2015; Karacosta et al. 2019; Brown et al. 2021). Thus, EMP is an excellent example of non-genetic heterogeneity where hybrid E/M cells can possess markers/traits of both E and M states within a predominant epithelial or mesenchymal cell population (Grosse-Wilde et al. 2015; Andriani et al. 2016; Celià-Terrassa et al. 2018).

A major reason enabling non-genetic heterogeneity in EMP has been multistability in underlying regulatory networks (Steinway et al. 2015; Font-Clos et al. 2018; Watanabe et al. 2019), which can often introduce asymmetry in the ‘paths’ taken by cells during EMT vs. those taken during MET. Indeed, transcriptional profiling of metastatic prostate cancer reveals that the expression profiles of cells at various timepoints are not just the reverse of those seen when cells underwent EMT (Stylianou et al. 2019). Similar patterns of hysteresis were seen in proteomics of lung cancer cells (Karacosta et al. 2019). Therefore, despite much investigation, we do not have a unique EMP signature that can be applied in a pan-cancer manner to identify whether cells are undergoing EMT or MET at a given timepoint and, by extension, a signature that can estimate the metastatic potential of cells.

Non-genetic heterogeneity has also been reported for a trait connected with EMT-Cancer Stem Cells (CSCs). CSCs were earlier thought to occupy the apex of cell differentiation (i.e., hierarchical model), but recent evidence has shown that CSCs and non-CSCs can interconvert among one another at both molecular and functional levels (Tang 2012; Thankamony et al. 2020). Moreover, CSCs can have multiple categories with varied EMT status: the CD44+/CD24− CSCs in breast cancer are more mesenchymal-like, while the
ALDH+ and CD44+ CD24+ ones map to a hybrid E/M phenotype (Liu et al. 2014; Colacino et al. 2018; Bocci et al. 2019a; Asadullah et al. 2021). Such subpopulations may stochastically transition among one another, suggesting that the tumorigenic potential of cancer cells is strongly associated with intrinsic plasticity rather than CSC multipotency per se (Dirkse et al. 2019). Similarly, in lung cancer, different subpopulations (holoclone, paraclone and meroclone) displayed variable tumour initiation capacity and EMT traits (Tièche et al. 2018). They maintained distinct morphology during short-term culture and displayed distinct markers and RNA expression profiles. While holoclones displayed the maximal epithelial trait, the paraclones displayed the most mesenchymal one, with merocloners being intermediate. Moreover, holoclones showed the highest and paraclones the lowest tumour-initiation capacity in vivo. On the other hand, paraclones showed the highest and holoclones had the lowest drug resistance features (Tièche et al. 2018). Subpopulations of CSCs have also been seen in squamous cell carcinoma (Biddle et al. 2011, 2016) and colorectal cancer (Hirata et al. 2019) among others.

Another axis connected to EMT that has growing evidence of phenotypic plasticity and heterogeneity is that of drug resistance. Of course, resistance can emerge due to de novo existing mutations in a subpopulation of cells and/or additional mutations gained during therapeutic assault, but non-genetic factors can play a role in driving drug resistance too (Salgia and Kulkarni 2018; Marine et al. 2020; Rebecca and Herlyn 2020). For instance, a partial or full EMT can drive ER+ breast cancer cells into resistance to tamoxifen and/or docetaxel; intriguingly, tamoxifen-resistant cells tend to be more mesenchymal than their sensitive counterparts, indicating a mutual causal connection between these axes (Prieto-Vila et al. 2019; Sahoo et al. 2021a). Similarly, non-genetic heterogeneity may provide a mechanism to adapt to drug treatment. By using quantitative proteomics and computational modeling, it was shown that immediately after exposure to RAF/MEK inhibitors such as vemurafenib, the BRAFV600E melanoma ‘persister’ cells show adaptive changes involving brief pulsatile reactivation of the MAPK pathway which can activate ERK signaling in neighbouring cells too. These pulses enable ‘persister’ cells to escape cell cycle arrest and sustain long-term resistance at a non-genetic level. This study provides mechanistic detail of the role of non-genetic heterogeneity in emergence of drug resistance in a genetically identical population (Gerosa et al. 2020). Additional analysis of drug-tolerant ‘persisters’ in melanoma has indicated how vemurafenib treatment can trigger cell-state transitions into a more undifferentiated phenotype which is therapeutically resilient (Su et al. 2017, 2019; Pillai and Jolly 2021). Such transitions are often reversible, as seen for EMT (Tripathi et al. 2020), thus enabling resumption of growth upon drug removal. Also, these drug-tolerant ‘persisters’ can serve as a reservoir of cells some of which may acquire additional mutations at prolonged timescales, leading to ‘stabilization’ of the drug-resistant phenotype, as seen in lung cancer cells treated with gefitinib (Ramirez et al. 2016).

The concept of persisters was initially reported in bacterial populations which, when exposed to antibiotic drugs, show differential killing rates such that the majority of bacterial cells (drug-sensitive) show fast killing rates and a steep decrease in their survival, while a small fraction of cells show slow killing with relatively slower decrease in their survival (Brauner et al. 2016; Rossi et al. 2019). This biphasic time-kill curve pinpointed the existence of a small fraction of cells called ‘persisters’, which, when isolated and grown in drug-free medium, repopulated the initial bacterial population consisting of drug-sensitive and persister cells, indicating phenotypic switching (Balaban et al. 2004) rather than inherited genetic mutations (Moyed and Bertrand 1983). Persister cells may arise from dormant bacterial cells even before exposure to antibiotics as suggested from single-cell and flow cytometry studies (Harms et al. 2016; Rossi et al. 2019), indicating the idea of bet-hedging, an evolutionary strategy to maximize fitness and survival of clonal population in dynamic environments by incorporating phenotypic heterogeneity (Veening et al. 2008a, b). Moreover, it indicates that within a clonal population, cells can have different interconvertible subpopulations, indicating bistability in biological systems (Feng et al. 2014; Jolly et al. 2018a).

Reinforcing observations are reported in luminal breast cancer using single-cell RNA sequencing, where a subpopulation of ‘pre-adapted’ cells, with reduced levels of estrogen receptor (ERα) and increased properties of quiescence and migration, undergo transcriptional reprogramming upon drug treatment and gather copy number changes to gain long-term resistance to endocrine therapy (Hong et al. 2019). Another study investigating the mechanism of drug resistance in triple-negative breast cancer has shown that after treatment with doxorubicin combined with cyclophosphamide, the residual tumour cells maintained the sub-clonal architecture of an untreated tumour; however, their transcriptomic, proteomic and histological profiles were different from that of the untreated tumour profiles. Once the drug treatment was stopped, residual tumours gave rise to drug-sensitive tumours with similar expression profiles as that of the
untreated tumour, indicating reversible chemotherapy tolerance (Echeverria et al. 2019). Similar analysis of trajectories of escapes from ALK inhibitor treatment was seen in NSCLC, where both genetic and non-genetic mechanisms contributed to gradual adaptation and gained resistance (Vander Velde et al. 2020).

Considered together, emerging evidence along multiple axes of cellular plasticity – EMT, CSCs and drug resistance – strongly endorses the role that non-genetic heterogeneity and consequent possible cell adaptation trajectories can play in facilitating tumour survival, therapy resistance and relapse.

5. Sources of non-genetic heterogeneity

What are the mechanisms that can result in non-genetic heterogeneity within a clonal population of cells as observed in microorganisms and cancer cells, as well as during embryonic development of a metazoan? Here, we briefly review some canonical sources of non-genetic heterogeneity in different scenarios, and their implications.

5.1 Epigenetic regulation and transcriptional noise

Besides genomic changes, some changes in chromatin such as covalent modification of chromatin components (DNA methylation and histone modification) can be inherited (Gerlinger et al. 2012). DNA hypermethylation and hypomethylation have been extensively studied in cancer and may result in the inactivation of tumour suppressor genes or activation of oncogenes, respectively (Feinberg et al. 2016; Kazanets et al. 2016). Recently, using single-cell RNA sequencing of naïve and drug-resistant acute myeloid leukemia (AML) patient samples, the occurrence of non-genetic resistance to BET inhibitor – driven by epigenetic mechanisms and transcriptional plasticity – was observed (Bell et al. 2019). Similarly, epigenetic changes can drive reversible drug tolerance observed in non-small-cell lung cancer cells (Sharma et al. 2010). These studies highlight the role of chromatin changes in non-genetic adaptation and pinpoint the compensatory mechanisms used by cancer cells to survive therapeutic attacks (Bell et al. 2019). Such chromatin-level changes, especially those including pioneer transcription factors, can alter the transcriptional landscape by mediating access to the corresponding promoter and/or enhancer regions.

Transcriptional processes are inherently stochastic, i.e., the production of mRNA and turnover rate of mRNA and protein can produce gene expression noise, which arises due to random binding of transcription factors to the gene promoter (Mcadams and Arkin 1997; Raser and O’Shea 2004). Non-continuous transcription may fluctuate the promoter between an ‘ON’ state (which results in a transcription burst, producing mRNA transcripts at a high rate) and an ‘OFF’ state (which pauses transcription) (Raj et al. 2006; Singh et al. 2012; Kumar et al. 2015; Friedrich et al. 2019). The amount of RNA produced from a particular gene depends upon the frequency, amplitude and duration of the transcription burst which may be specific for a particular cell depending upon extrinsic noise such as concentration and availability of general transcription factors (Elowitz et al. 2002). The frequency and size of a transcriptional burst also depends upon the composition of the gene promoter which can modulate binding affinities and trans-acting factor concentrations (Hendy et al. 2017). For example, it is shown that the presence of a TATA box in the gene promoter is associated with higher noise (Hornung et al. 2012; Tantale et al. 2016). In addition, transcription bursting also depends upon enhancer–promoter interaction and enhancer strength (Fukaya et al. 2016). Enhancers control the spatial and temporal expression of genes by recruiting gene-specific transcription factors (Buecker and Wysocka 2012). Similar to the rate of synthesis, the rate of degradation of mRNA also influences gene expression noise (Baudrimont et al. 2019). Thus, gene expression noise may differ drastically between genetically identical cells depending on multiple factors (Balázsi et al. 2011; Urban and Johnston 2018) which may result in phenotypic variations in cells (Brock et al. 2009) by imparting variability and/or memory for protein levels in a cell (Sigal et al. 2006).

Transcriptional noise has been implicated as a source for cell-fate decision-making in yeast (Blake et al. 2006), bacteria (Süel et al. 2006) and many mammalian systems (Moris et al. 2016). More recently, it has been shown to influence cancer progression. In leukemia, depletion of acetyl-transferase KAT2A enhances transcriptional bursting and variability, depletes leukemic stem-like cells and delays disease progression (Domingues et al. 2020).

5.2 Conformational noise

Intrinsically disordered proteins (IDPs) are proteins, which, unlike many other proteins, lack a well-defined 3D structure either locally or throughout the protein and
display a high degree of flexibility which can confer ‘conformational noise’ due to promiscuity in their interactions (Mahmoudabadi et al. 2013). This ‘conformational noise’ may manifest differently in different cells, depending on the fluctuations that such IDPs may go through in individual cells. Due to their high conformational flexibility, IDPs serve as a determinant of protein activity and can often be present as hub proteins in biochemical networks (Haynes et al. 2006; Patil et al. 2010). IDPs display faster binding/unbinding rates with their ligands and may undergo transition from disordered to ordered states upon binding with their partner or post-translational modification, and thus may amplify promiscuity and bring stochasticity in interactions in biochemical networks (Chakrabortee et al. 2010; Bah et al. 2015; Jolly et al. 2018a; Lin et al. 2018). This promiscuity may lead to dynamic rewiring of protein–protein interaction networks, thus potentially impinging on transcriptional and translational noise also. For example, many drivers of EMT such as ZEB1 and OVOL1/2 (Saxena et al. 2020) have been predicted to contain intrinsically disordered regions (Mooney et al. 2016), adding to the long list of oncogenes and/or tumour suppressor genes where IDP regions have been reported consistently (Lin et al. 2019). Depending on which proteins ZEB1 and/or OVOL1/2 stably interact with in a given time interval, the phenotypic outcome of cells in a population may be varied.

For example, an IDP associated with prostate cancer—Prostate Associated Gene 4 (PAGE4)—has been implicated in phenotypic heterogeneity (Kulkarni et al. 2017; Singh et al. 2021). PAGE4 is phosphorylated by two kinases, namely, Homeodomain Interacting Protein Kinase 1 (HIPK1) and CDC-Like Kinase 2 (CLK2). PAGE4 activates the Activator Protein-1 (AP-1). HIPK1-PAGE4 has a stronger affinity to AP-1 when compared with CLK2-PAGE4. Experimental studies and mathematical modeling have shown that as a result of differential phosphorylation of PAGE4 which leads to an altered AP-1/androgen receptor (AR) regulatory circuit, prostate cancer cells can have a spectrum of phenotypes with varying sensitivity to the standard-of-care androgen deprivation therapy (ADT). Furthermore, the fact that a majority of transcription factors are intrinsically disordered (Staby et al. 2017; Niklas et al. 2018; Tsafou et al. 2018; Zhang and Tjian 2018) lends further credence to the argument.

5.3 Tumour microenvironment

The tumour microenvironment shows a high degree of heterogeneity in terms of angiogenesis which modulates oxygen and nutrient availability, composition of stromal and immune cells, endothelial cell density and extracellular matrix composition (Quail and Joyce 2013; Saxena and Jolly 2019). Hypoxia, for instance, confers certain phenotypes on tumour cells present in hypoxic regions, such as stemness (Louie et al. 2010), EMT (Liu et al. 2017) and chemoresistance (Chen et al. 2015). Similarly, varying spatial localization of stromal cells and immune cells with respect to cancer cells can govern the autocrine/paracrine impact they can have on each other. For example, cancer-associated fibroblasts (CAFAs) have been shown to expand the breast cancer stem cell population by secreting prostaglandin (PGE2) and IL-6 (Rudnick et al. 2011), to promote migration and invasion mediated by high expression of COX-2 in nasopharyngeal carcinoma (Zhu et al. 2020) and to facilitate neo-angiogenesis in hepatocellular cancer via placental growth factor (Liu et al. 2019). Further, not all fibroblasts are pro-tumour; a subset of them, as identified via single-cell analysis, can also support anti-tumour immunity (Hutton et al. 2021). Similarly, neutrophils may exert pro- or anti-tumour effects and these subpopulations may also interconvert among one another (Furumaya et al. 2020), reminiscent of macrophage phenotypic heterogeneity exhibited along the M1-M2 axis. Also, feedback loops formed between immune cells and/or cancer cells of varying phenotypes can allow for dynamic phenotypic composition in a tumour, thus influencing its prognosis (Li et al. 2019). Thus, both cell-autonomous (transcriptional, conformational noise and epigenetic changes) and non-cell-autonomous (tumour–stroma interactions) can amplify non-genetic heterogeneity in cancer.

6. Mathematical models to understand non-genetic heterogeneity

In 1957, Waddington proposed an epigenetic landscape model to explain how a pluripotent stem cell can differentiate into multiple lineages represented as valleys (Waddington 1957) (figure 2A). From a dynamical systems theory perspective, these valleys represent ‘attractors’ in a high-dimensional landscape. These ‘attractors’ correspond to stable gene expression patterns defining a phenotype, and for a given gene regulatory network (GRN), these ‘attractors’ can be identified by simulating their emergent non-linear dynamics (Wang et al. 2011; Ferrell 2012; Jia et al. 2017). Many GRNs driving phenotypic plasticity are multistable in nature, i.e., they have multiple attractors.
Thus, cells having these GRNs can acquire more than one phenotype, and can also switch among them under the influence of biological perturbations or noise (Wooten and Quaranta 2017; Li and Balazsi 2018; Sahoo et al. 2020) (figure 2B). Considering the large number of genes in eukaryotes and their web of complex interactions, there can be many possible ‘attractors’, but the conditions imposed by the underlying network topology can restrain the ‘solution space’ to enable acquisition of only a few attractors. These conditions are akin to those imposed by energy minimization principles during protein folding, such that only a limited number of protein configurations are achieved, starting from a given amino acid sequence. Thus, cells can be postulated to traverse in a landscape where each ‘attractor’ corresponds to stable phenotypes and has a specific basin of attraction (Agozzino et al. 2020). During cancer progression, various genomic changes may alter access to various cell types/attractors, thus modifying the underlying landscape (Huang et al. 2009).

Non-genetic heterogeneity in a clonal population indicates the presence of multiple stable states (or attractors) of the same GRN. Each attractor can have sub-attractors which make its surface rugged (Chang et al. 2008); in other words, each ‘macro-state’ can have multiple ‘micro-states’. Owing to various cell-intrinsic and cell-extrinsic factors underlying biological noise (Balázsi et al. 2011), stochastic perturbations may result in the establishment of outlier or edge cells which are present near the borders of a given basin of attraction (Brock et al. 2009; Gopalan et al. 2021). Similarly, during metastasis, the presence of hybrid epithelial/mesenchymal (E/M) phenotype(s) can accelerate disease progression due to their enhanced ability to switch to more epithelial or more mesenchymal ones (Ruscetti

Figure 2. Waddington landscape and phenotypic plasticity. (A) Schematic of the Waddington’s landscape showing multiple different paths that the cells can take during embryonic development (each ball represents a differentiating cell; each valley represents a phenotype). (B) (Left) In case of multistability, cells can switch back and forth among various phenotypes (valleys). (Right) Biological noise due to various sources (transcriptional, conformational, etc.) can drive phenotypic plasticity.
et al. 2016; Goetz et al. 2020; Jolly et al. 2018b). Further, the presence of different subpopulations in multiple cancers is well established. For instance, using a Markov model of stochastic cell transition, it was shown that a subpopulation of cells returns to equilibrium phenotypic proportions with time, and thus breast cancer stem cells arise from non-cancer stem-like cells de novo, highlighting the role of stochasticity in enabling phenotypic heterogeneity in a clonal cell population (Gupta et al. 2011). Another study in small-cell lung cancer (SCLC) used Boolean modeling of the underlying GRN to show that the network dynamics can stabilize neuro-endocrine/epithelial (NE) or non-neuroendocrine/mesenchymal-like (ML) phenotypes which act as attractors. Additionally, they also found that when NE and ML cells were treated with cytotoxic drugs, these cells converged towards a hybrid state displaying surface markers of both NE and ML, possibly as a strategy to escape the cytotoxic effects of the treatment (Udyavar et al. 2017). Similarly, in melanoma, identification and simulation of an underlying GRN enabled four different attractors which mapped to the four distinct phenotypes reported experimentally – proliferative, neural crest-like, intermediate/transitory and undifferentiated (Rambow et al. 2018; Pillai and Jolly 2021). This computational analysis could also recapitulate the cell-state transition trajectory observed experimentally upon treatment with vemurafenib through single-cell analysis (Su et al. 2019), offering a platform to identify novel perturbations that can enrich or deplete certain phenotypes.

Mathematical models have helped construct the landscapes of cell-state transitions associated with non-genetic heterogeneity in cancer, such as those for EMT, CSCs, metabolic reprogramming and drug resistance, using both deterministic and stochastic approaches (such as Gillespie simulations; Gillespie 2007) (Font-Clos et al. 2018; Kang et al. 2019; Sarkar et al. 2019; Lang et al. 2021; Sahoo et al. 2021a). Particularly, in EMT, the concept of partial EMT, also referred as hybrid E/M phenotypes, has been largely championed by mathematical models decoding the emergent dynamics of highly inter-connected mutually inhibitory feedback loops involving miR-200/ZEB and miR-34/SNAIL (Lu et al. 2013; Tian et al. 2013). These models predicted that, contrary to previous assumptions, hybrid E/M states are not mere intermediates or ‘metastable’ states, but are, instead, stable phenotypes that cells can acquire. Further, such mechanistic mathematical models have made experimentally testable predictions about factors stabilizing hybrid E/M states. Validating those predictions in vitro led to identification of ‘phenotypic stability factors’ (PSFs) – GRHL2, OVOL1/2, NRF2, NUMB, NFATc, among others (Hong et al. 2015; Biswas et al. 2019; Bocci et al. 2019b; Pastushenko and Blanpain 2019; Subbalakshmi et al. 2020). Mathematical models have also elucidated the cell-state transition dynamics upon EMT induction, identifying multiple ‘micro-states’ and/or hybrid E/M phenotypes that cells acquire en route to EMT in a dose- and/or time-dependent manner (Zhang et al. 2014; Steinway et al. 2015; Font-Clos et al. 2018; Celià-Terrassa et al. 2018; Sha et al. 2020; Deshmukh et al. 2021). Predictions made by mathematical models for coupled EMT-stemness networks (Jolly et al. 2014) about the high tumour-initiating potential of hybrid E/M phenotypes have also been recently validated in vitro and in vivo (Bierie et al. 2017; Pastushenko et al. 2018; Kröger et al. 2019). For instance, the presence of hybrid E/M cells was associated with the worst survival in breast cancer patients and enriched for stem-like cells in different types of breast cancer cell lines with properties such as increased mammosphere formation and higher ALDH1 levels (Grosse-Wilde et al. 2015). Another insight gained by mathematical models of EMT has been that various positive feedback loops in a GRN drive plasticity among epithelial, mesenchymal or hybrid E/M phenotypes (Hari et al. 2020). Indeed, breast cancer cells with the miR-200/ZEB positive feedback loop perturbed via CRISPR had reduced metastatic potential in vivo (Celià-Terrassa et al. 2018), suggesting that mathematical models can not only elucidate the dynamical principles of non-genetic heterogeneity and cell-fate transitions in cancer, but also pinpoint specific therapeutic vulnerabilities to be tested.

The functional role of feedback loops and gene expression noise in enabling drug resistance was recently investigated using synthetic gene network circuits to deconvolute noise from the mean expression of the puromycin-resistance gene with inducible positive and negative feedback loops in Chinese hamster ovary cells. This study demonstrated that the greater noise emerging from the positive feedback loop increased drug resistance at higher concentrations of puromycin, but at lower drug concentrations, it delayed long-term adaptation. Further, a positive correlation between low noise as a result of lower concentrations, it delayed long-term adaptation. Further, a positive correlation between low noise as a result of negative feedback circuits and mutational adaptation driving stable drug resistance was observed (Farquhar et al. 2019). The cross-talk of various positive and negative feedback loops in a cell can therefore influence its sensitivity to various chemotherapeutic assaults, leading to fractional killing (Spencer et al. 2009; Pack et al. 2016; Miura et al. 2018; Guinn et al. 2020). Such non-genetic heterogeneity in a clonal cell population, upon the influence of drug-induced reprogramming, can lead to a rare and stably resistant subpopulation of cells (Shaffer et al.
Population dynamics models capturing such reversible (non-genetic) and/or stable (genetic) resistance scenarios can suggest combinatorial and/or sequential therapeutic strategies to prevent or delay the emergence of tumour (re)growth (Gunnarsson et al. 2020; Cassidy et al. 2021; Sahoo et al. 2021a).

7. Conclusion

Non-genetic heterogeneity can confer fitness advantage to a clonal population of cancer cells during metastasis, acquisition of therapy resistance and tumour progression. Such heterogeneity can arise from various sources of biological noise within a cell as well as due to multistable dynamics of various underlying networks. Integrated and iterative mathematical–experimental approaches have been instrumental in identifying the sources and implications of non-genetic heterogeneity in cancer. Developing therapeutic strategies which can target the sources of such heterogeneity in isogenic cancer cells may result in higher efficacy in preventing metastasis and tumour progression.

Glossary

Attractors: attractors usually refer to discrete stable steady states of a dynamical system. In the context of cellular decision-making, attractors can be considered as ‘valleys’ seen in the metaphorical Waddington landscape. They denote the different ‘phenotypes’, or states, that the cell population can attain during differentiation. Switching among attractors is often called trans-differentiation or de-differentiation, depending on the attractors and trajectories involved in such switching.

Noise: noise refers to stochastic effects in various cellular processes such as transcription and translation, which can drive cell-to-cell (phenotypic) heterogeneity. In cells, noise is usually categorized into intrinsic (noise due to stochastic effects that influence the mRNA and protein levels of a given gene in a cell) and extrinsic (noise due to factors that can influence the mRNA and protein levels of multiple genes simultaneously, including extracellular factors such as the microenvironment).

Variability: differences between cells, individual organisms, or groups of organisms of a given species caused either by genetic differences (genotypic variability) or by the effect of environmental factors on expression of genetic players (phenotypic variability). It is often quantified using the Fano factor, defined as the ratio of variance to the mean.

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References

Andriani F, Bertolini G, Facchinetti F, et al. 2016 Conversion to stem-cell state in response to microenvironmental cues is regulated by balance between epithelial and mesenchymal features in lung cancer cells. Mol. Oncol. 10 253–271

Agoozino L, Balázsi G, Wang J and Dill KA 2020 How do cells adapt? Stories told in landscapes. Annu. Rev. Chem. Biomol. Eng. 11 155–182

Asadullah A, Kumar S, Saxena N, et al. 2021 Combined heterogeneity in cell size and deformability promotes cancer invasiveness. J. Cell Sci. 134 jcs250225

Bah A, Vernon RM, Siddiqi Z, et al. 2015 Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. Nature 519 106–109

Balaban NQ, Merrin J, Chait R, Kowalik L and Leibler S 2004 Bacterial persistence as a phenotypic switch. Science 305 1622–1625

Balázsi G, Van Oudenaarden A and Collins JJ 2011 Cellular decision making and biological noise: from microbes to mammals. Cell 144 910–925

Barcellos-Hoff MH and Ravani SA 2000 Irradiated mammary gland stroma promotes the expression of tumourigenic potential by unirradiated epithelial cells. Cancer Res. 60 1254–1260

Barclay WW, Woodruff RD, Hall MC and Cramer SD 2005 A system for studying epithelial-stromal interactions reveals distinct inductive abilities of stromal cells from benign prostatic hyperplasia and prostate cancer. Endocrinology 146 13–18

Barzgar Borough N, Sajjadian F, Jalilzadeh N, Shafiei H and Velaei K 2021 Understanding breast cancer heterogeneity through non-genetic heterogeneity. Breast Cancer 28 777–791

Baudrimont A, Jaquet V, Wallerich S, Voegeli S and Becksei A 2019 Contribution of RNA degradation to intrinsic and extrinsic noise in gene expression. Cell Rep. 26 3752–3761

Beadle GW and Tatum EL 1941 Genetic control of biochemical reactions in neurospora. Proc. Natl. Acad. Sci. USA 27 499–506
Bell CC, Fennell KA, Chan Y-C, et al. 2019 Targeting enhancer switching overcomes non-genetic drug resistance in acute myeloid leukaemia. Nat. Commun. 10 2723
Bell CC and Gilan O 2020 Principles and mechanisms of non-genetic resistance in cancer. Br. J. Cancer 122 465–472
Bering C, Takemoto R, Satyamoorthy K, et al. 2004 Induction of melanoma phenotypes in human skin by growth factors and ultraviolet B. Cancer Res. 64 807–811
Bhatia S, Wang P, Toh A and Thompson EW 2020 New insights into the role of phenotypic plasticity and EMT in driving cancer progression. Front. Mol. Biosci. 7 1–18
Biddle A, Gammon L, Liang X, Costea DE and Mackenzie IC 2016 Phenotypic plasticity determines cancer stem cell therapeutic resistance in oral squamous cell carcinoma. EBioMedicine 4 138–145
Biddle A, Liang X, Gammon L, et al. 2011 Cancer stem cells in squamous cell carcinoma switch between two distinct phenotypes that are preferentially migratory or proliferative. Cancer Res. 71 5317–5326
Bierie B, Pierce SE, Kroeger C, et al. 2017 Integrin-β4 identifies cancer stem cell-enriched populations of partially mesenchymal carcinoma cells. Proc. Natl. Acad. Sci. USA 114 E2337–2346
Biswas K, Jolly MK and Ghosh A 2019 Stability and mean residence times for hybrid epithelial/mesenchymal phenotype. Phys. Biol. 16 025003
Blake W, Balázs G, Kohanski M, et al. 2006 Phenotypic consequences of promoter-mediated transcriptional noise. Mol. Cell 24 855–865
Bocci F, Gearhart-Serna L, Boaretto M, et al. 2019 Toward understanding cancer stem cell heterogeneity in the tumour microenvironment. Proc. Natl. Acad. Sci. USA 116 148–157
Bocci F, Jolly MK, Tripathi SC, et al. 2017 Numb prevents a complete epithelial-mesenchymal transition by modulating Notch signalling. J. R. Soc. Interface 14 20170512
Bocci F, Mandal S, Tejaswi T and Jolly MK 2021 Investigating epithelial-mesenchymal heterogeneity of tumours and circulating tumour cells with transcriptomic analysis and biophysical modeling. Comput. Syst. Oncol. 1 e1015
Bocci F, Tripathi SC, Vlček MSA, et al. 2019b NRF2 activates a partial epithelial-mesenchymal transition and is maximally present in a hybrid epithelial/mesenchymal phenotype. Integr. Biol. 11 251–263
Boumahdi S and de Sauvage FJ 2020 The great escape: tumour cell plasticity in resistance to targeted therapy. Nat. Rev. Drug Discov. 19 39–56
Brabletz S and Brabletz T 2010 The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer? EMBO Rep. 11 670–677
Brandman O and Meyer T 2008 Feedback loops shape cellular signals in space and time. Science 322 390–395
Brauner A, Fridman O, Gefen O and Balaban NQ 2016 Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat. Rev. Microbiol. 14 320–330
Brock A, Chang H and Huang S 2009 Non-genetic heterogeneity—a mutation-independent driving force for the somatic evolution of tumours. Nat. Rev. Genet. 10 336–342
Brown MS, Abdollahi B, Wilkins OM, et al. 2021 Dynamic plasticity within the EMT spectrum, rather than static mesenchymal traits, drives tumour heterogeneity and metastatic progression of breast cancers. bioRxiv. https://doi.org/10.1101/2021.03.17.434993
Buecker C and Wysocka J 2012 Enhancers as information integration hubs in development: lessons from genomics. Trends Genet. 28 276–284
Bussard KM, Boulanger CA, Booth BW, Bruno RD and Smith GH 2010 Reprogramming human cancer cells in the mouse mammary gland. Cancer Res. 70 6336–6343
Cameron MD, Schmidt EE, Kerkvliet N, et al. 2000 Temporal progression of metastasis in lung: Cell survival, dormancy, and location dependence of metastatic inefficiency. Cancer Res. 60 2541–2546
Cassidy T, Nichol D, Robertson-Tessi M, Craig M and Anderson ARA 2021 The role of memory in non-genetic inheritance and its impact on cancer treatment resistance. PLoS Comput. Biol. 17 e1009348
Celià-Terrassa T, Bastian C, Liu DD, et al. 2018 Hysteresis control of epithelial-mesenchymal transition dynamics conveys a distinct program with enhanced metastatic ability. Nat. Commun. 9 5005
Celià-Terrassa T and Jolly MK 2020 Cancer stem cells and epithelial-to-mesenchymal transition in cancer metastasis. Cold Spring Harb. Perspect. Med. 10 a036905
Celià-Terrassa T and Kang Y 2016 Distinctive properties of metastasis-initiating cells. Genes Dev. 30 892–908
Celià-Terrassa T, Meca-Cortés Ó, Mateo F, et al. 2012 Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumour-initiating cells. J. Clin. Investig. 122 1849–1868
Chakrabortee S, Meersman F, Kaminski Schierle GS, et al. 2010 Catalytic and chaperone-like functions in an intrinsically disordered protein associated with desiccation tolerance. Proc. Natl. Acad. Sci. USA 107 16084–16089
Chang HH, Hemberg M, Barahona M, Ingber DE and Huang S 2008 Transcriptional-wide noise controls lineage choice in mammalian progenitor cells. Nature 453 544–547
Chen L, Gibbons DL, Goswami S, et al. 2014 Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoural immunosuppression. Nat. Commun. 5 5241
Chen WL, Wang CC, Lin YJ, Wu CP and Hsieh CH 2015 Cycling hypoxia induces chemoresistance through the
activation of reactive oxygen species-mediated B-cell lymphoma extra-long pathway in glioblastoma multiforme. *J. Transl. Med.* **13** 389

Chouabi S, Janbi B, Tittarelli A, Eggermont A and Thiery JP 2014 Tumour plasticity interferes with anti-tumour immunity. *Crit. Rev. Immunol.* **34** 91–102

Cieply B, Riley P IV, Pifer PM, *et al.* 2012 Suppression of the epithelial-mesenchymal transition by grainyhead-like-2. *Cancer Res.* **72** 2440–2453

Colacino JA, Azizi E, Brooks MD, *et al.* 2018 Heterogeneity of human breast stem and progenitor cells as revealed by transcriptional profiling. *Stem Cell Rep.* **10** 1596–1609

Cook DP and Vanderhyden BC 2020 Context specificity of the EMT transcriptional response. *Nat. Commun.* **11** 2142

Dalerba P, Kalisky T, Sahoo D, *et al.* 2011 Single-cell dissection of transcriptional heterogeneity in human colon tumours. *Nat. Biotechnol.* **29** 1120–1127

Davidson CJ and Surette MG 2008 Individuality in Bacteria. *Annu. Rev. Genet.* **42** 253–268

Deshmukh AP, Vasaikar SV, Tomczak K, *et al.* 2021 Identification of EMT signaling cross-talk and gene regulatory networks by single-cell RNA sequencing. *Proc. Natl. Acad. Sci. USA* **118** e2102050118

Devaraj V and Bose B 2019 Morphological state transition dynamics in EGF-induced epithelial to mesenchymal transition. *J. Clin. Med.* **8** 911

Dhar R, Missarova AM, Lehner B and Carey LB 2019 Single cell functional genomics reveals the importance of mitochondria in cell-to-cell phenotypic variation. *eLife* **8** e38904

Dirkse A, Golebiewska A, Buder T, *et al.* 2019 Stem cell- associated heterogeneity in Glioblastoma results from intrinsic tumour plasticity shaped by the microenvironment. *Nat. Commun.* **10** 1787

Domingues AF, Kulkarni R, Giotopoulos G, *et al.* 2020 Loss of KAT2A enhances transcriptional noise and depletes acute myeloid leukemia stem-like cells. *eLife* **9** e51754

Dongre A, Rashidian M, Reinhardt F, *et al.* 2017 Epithelial-to-mesenchymal transition contributes to immunosuppression in breast carcinomas. *Cancer Res.* **77** 3982–3989

Duda DG, Duyverman AMMJ, Kohno M, *et al.* 2010 Malignant cells facilitate lung metastasis by bringing their own soil. *Proc. Natl. Acad. Sci. USA* **107** 21677–21682

Echeverria GV, Ge Z, Seth S, *et al.* 2019 Resistance to neoadjuvant chemotherapy in triple-negative breast cancer mediated by a reversible drug-tolerant state. *Sci. Transl. Med.* **11** eaa0936

Elowitz MB, Levine AJ, Siggia ED and Swain PS 2002 Stochastic gene expression in a single cell. *Science* **297** 1183–1186

Evans TD and Zhang F 2020 Bacterial metabolic heterogeneity: origins and applications in engineering and infectious disease. *Curr. Opin. Biotechnol.* **64** 183–189

Farquhar KS, Charlebois DA, Szenk M, *et al.* 2019 Role of network-mediated stochasticity in mammalian drug resistance. *Nat. Commun.* **10** 2766

Feinberg AP, Koldobskiy MA and Gondor A 2016 Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. *Nat. Rev. Genet.* **17** 284–299

Feng J, Kessler DA, Ben-Jacob E and Levine H 2014 Growth feedback as a basis for persistor bistability. *Proc. Natl. Acad. Sci. USA* **111** 544–549

Ferguson B, Handoko HY, Mukhopadhyay P, *et al.* 2019 Different genetic mechanisms mediate spontaneous versus UVR-induced malignant melanoma. *eLife* **8** e42424

Ferrell JE 2012 Bistability, bifurcations, and waddington’s epigenetic landscape. *Curr. Biol.* **22** R458–R466

Fierst JL 2011 A history of phenotypic plasticity accelerates adaptation to a new environment. *J. Evolut. Biol.* **24** 1992–2011

Font-Clos F, Zapperi S and La Porta CAM 2018 Topography of epithelial–mesenchymal plasticity. *Proc. Natl. Acad. Sci. USA* **115** 5902–5907

Friedrich D, Friedel L, Finzel A, *et al.* 2021 Transcriptional bursting. *Cell* **166** 358–368

Furumaya C, Martinez-Sanz P, Bouti P, Kuijpers TW and Matlung HL 2020 Plasticity in pro- and anti-tumour activity of neutrophils: shifting the balance. *Front. Immunol.* **11** 2100

Genovese G, Kahler AK, Handsaker RE, *et al.* 2014 Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* **371** 2477–2487

George JT, Jolly MK, Xu S, Somarelli JA and Levine H 2017 Survival outcomes in cancer patients predicted by a partial EMT gene expression scoring metric. *Cancer Res.* **77** 6415–6428

Gerlinger M, Rowan AJ, Horswell S, *et al.* 2012 Intratumour heterogeneity and branched evolution revealed by multi-region sequencing. *N. Engl. J. Med.* **366** 883–892

Gerosa L, Chidley C, Froehlich F, *et al.* 2020 Receptor-driven ERK pulses reconfigure MAPK signaling and enable persistence of drug-adapted BRAF-Mutant melanoma cells. *Cell Syst.* **11** 478–494.e9

Gillespie DT 2007 Stochastic simulation of chemical kinetics. *Annu. Rev. Phys. Chem.* **58** 35–55

Goetz H, Melendez-Alvarez JR, Chen L and Tian X-J 2020 A plausible accelerating function of intermediate states in cancer metastasis. *PLoS Comput. Biol.* **16** e1007682

Gopalan V, Singh A, Mehrabadi FR, *et al.* 2021 A transcriptionally distinct subpopulation of healthy acinar cells exhibit features of pancreatic progenitors and PDAC. *Cancer Res.* **81** 3958–3970

Goury-Sistla P, Nanjundiah V and Pande G 2012 Bimodal distribution of motility and cell fate in Dictostelium discoideum. *Int. J. Dev. Biol.* **56** 263–272
Greaves M and Maley CC 2012 Clonal evolution in cancer. Nature 481 306–313
Grosse-Wilde A, Fouquier d’ Herouei A, McIntosh E, et al. 2015 Stemness of the hybrid epithelial/mesenchymal state in breast cancer and its association with poor survival. PLoS One 10 e0126522
Guinn MT, Wan Y, Levovitz S, et al. 2020 Observation and control of gene expression noise: barrier crossing analogies between drug resistance and metastasis. Front. Genet. 11 586726
Gunnarsson EB, De S, Leder K and Foo J 2020 Understanding the role of phenotypic switching in cancer drug resistance. J. Theor. Biol. 490 110162
Gupta PB, Fillmore CM, Jiang G, et al. 2011 Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell 146 633–644
Gupta PB, Pastushenko I, Skibinski A, Blanpain C and Kuperwasser C 2019 Phenotypic plasticity: driver of cancer initiation, progression, and therapy resistance. Cell Stem Cell 24 65–78
Haas D, Ablin AR, Miller C, Zoger S and Matthay KK 1988 Hanahan D and Weinberg RA 2011 Hallmarks of cancer. Nature 24 65–78
Harms A, Maisonneuve E and Gerdes K 2016 Mechanisms of bacterial persistence during stress and antibiotic exposure. Science 354 aaf4268
Hayford CE, Tyson DR, Robbins CJ, et al. 2021 An in vitro model of tumour heterogeneity resolves genetic, epigenetic, and stochastic sources of cell state variability. PLoS Biol. 19 e3007979
Haynes C, Oldfield CJ, Ji F, et al. 2006 Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes. PLoS Comput. Biol. 2 e100
Hendy O, Campbell J, Weissman JD, Larson DR and Singer DS 2017 Differential context-specific impact of individual core promoter elements on transcriptional dynamics. Mol. Biol. Cell 28 3360–3370
Hirata A, Hatano Y, Niwa M, Hara A and Tomita H 2019 Heterogeneity in colorectal cancer stem cells. Cancer Prev. Res. 12 413–420
Hong SP, Chan TE, Lombardo Y, et al. 2019 Single-cell transcriptomics reveals multi-step adaptations to endocrine therapy. Nat. Commun. 10 3840
Hong T, Watanabe K, Ta CH, et al. 2015 An Ovol2-Zeb1 mutual inhibitory circuit governs bidirectional and multi-step transition between epithelial and mesenchymal states. PLoS Comput. Biol. 11 e1004569
Hornung G, Bar-Ziv R, Rosin D, et al. 2012 Noise-mean relationship in mutated promoters. Genome Res. 22 2409–2417
Hu Z, Artibani M, Alsaadi A, et al. 2020 The repertoire of serous ovarian cancer non-genetic heterogeneity revealed by single-cell sequencing of normal fallopian tube epithelial cells. Cancer Cell 37 226–242.e7
Huang RY-J, Wong MK, Tan TZ, et al. 2013 An EMT spectrum defines an anoikis-resistant and spheroidogenic intermediate mesenchymal state that is sensitive to e-cadherin restoration by a src-kinase inhibitor, saracatinib (AZD0530). Cell Death Dis. 4 e915
Huang S, Enberg I and Kauffman SA 2009 Cancer attractors: a systems view of tumours from a gene network dynamics and developmental perspective. Semin. Cell Dev. Biol. 20 869–876
Huh D and Paulsson J 2011 Non-genetic heterogeneity from stochastic partitioning at cell division. Nat. Genet. 43 95–100
Hutton C, Heider F, Blanco-Gomez A, et al. 2021 Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumour immunity. Cancer Cell 39 1227–1244.e20
Jia D, Jolly MK, Kulkarni P and Levine H 2017 Phenotypic plasticity and cell fate decisions in cancer: Insights from dynamical systems theory. Cancers 9 E70
Jia D, Park JH, Kaur H, et al. 2021 Towards decoding the coupled decision-making of metabolism and epithelial-to-mesenchymal transition in cancer. Br. J. Cancer 124 1902–1911
Jia W, Deshmukh A, Mani SA, Jolly MK and Levine H 2019 A possible role for epigenetic feedback regulation in the dynamics of the Epithelial-Mesenchymal Transition (EMT). Phys. Biol. 16 066004
Jolly MK, Huang B, Lu M, et al. 2014 Towards elucidating the connection between epithelial-mesenchymal transitions and stemness. J. R. Soc. Interface 11 20140962
Jolly MK, Jia D, Boareto M, et al. 2015 Coupling the modules of EMT and stemness: A tunable stemness window model. Oncotarget 6 25161–25174
Jolly MK, Kulkarni P, Weninger K, Orban J and Levine H 2018a Phenotypic plasticity, bet-hedging, and androgen independence in prostate cancer: role of non-genetic heterogeneity. Front. Oncol. 8 50
Jolly MK, Mani SA and Levine H 2018b Hybrid epithelial/mesenchymal phenotype(s): The ‘fittest’ for metastasis? Biochim. Biophys. Acta Rev. Cancer 1870 151–157
Kang X, Wang J and Li C 2019 Exposing the underlying relationship of cancer metastasis to metabolism and epithelial-mesenchymal transitions. iScience 21 754–772
Karacosta LG, Anchang B, Ignatiadis N, et al. 2019 Mapping lung cancer epithelial-mesenchymal transition states and trajectories with single-cell resolution. Nat. Commun. 10 5587
Karki P, Angardi V, Mier JC and Orman MA 2021 A transient metabolic state in melanoma persister cells mediated by chemotherapeutic treatments. bioRxiv 432154
Kasemeier-Kulesa JC, Teddy JM, Postovit LM, et al. 2008 Reprogramming multipotent tumour cells with the embryonic neural crest microenvironment. Dev. Dyn. 237 2657–2666
Kazanets A, Shorstova T, Hilmi K, Marques M and Witcher M 2016 Epigenetic silencing of tumour suppressor genes: Paradigms, puzzles, and potential. Biochim. Biophys. Acta Rev. Cancer 1865 275–288
Kennedy SR, Zhang Y and Risques RA 2019 Cancer-associated mutations but no cancer: insights into the early steps of carcinogenesis and implications for early cancer detection. Trends Cancer 5 531–540
Krebs AM, Mitschke J, Losada ML, et al. 2017 The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nat. Cell Biol. 19 518–529
Krimmell JD, Schmitt MW, Harrell MI, et al. 2016 Ultra-deep sequencing detects ovarian cancer cells in peritoneal fluid and reveals somatic TP53 mutations in noncancerous tissues. Proc. Natl. Acad. Sci. USA 113 6005–6010
Kröger C, Afeyan A, Mraz J, et al. 2019 Acquisition of a hybrid E/M state is essential for tumourigenicity of basal breast cancer cells. Proc. Natl. Acad. Sci. USA 116 7353–7362
Kulkarni P, Jolly MK, Jia D, et al. 2017 Phosphorylation-induced conformational dynamics in an intrinsically disordered protein and potential role in phenotypic heterogeneity. Proc. Natl. Acad. Sci. USA 114 E2644–E2653
Kumar N, Singh A and Kulkarni RV 2015 Transcriptional bursting in gene expression: analytical results for general stochastic models. PLoS Comput. Biol. 11 e1004292
Kvokackova B, Remsk J, Jolly MK and Soucek K 2021 Phenotypic heterogeneity of triple-negative breast cancer mediated by epithelial-mesenchymal plasticity. Cancers 13 2188
Lang J, Nie Q and Li C 2021 Landscape and kinetic path quantify critical transitions in epithelial-mesenchymal transition. Biophys. J. 120 4484–4500
Lee J, Lee J, Farquhar KS, et al. 2014 Network of mutually repressive metastasis regulators can promote cell heterogeneity and metastatic transitions. Proc. Natl. Acad. Sci. USA 111 E364–E373
Lewis AC and Kats LM 2021 Non-genetic heterogeneity, altered cell fate and differentiation therapy. EMBO Mol. Med. 13 e12670
Li C and Balazsi G 2018 A landscape view on the interplay between EMT and cancer metastasis. NPJ Syst. Biol. Appl. 4 34
Li S, Giardina DM and Siegal ML 2018 Control of nongenetic heterogeneity in growth rate and stress tolerance of Saccharomyces cerevisiae by cyclic AMP-regulated transcription factors. PLoS Genet. 14 e1007744
Li X, Jolly MK, George JT, Pienta KJ and Levine H 2019 Computational modeling of the crosstalk between macrophage polarization and tumour cell plasticity in the tumour microenvironment. Front. Oncol. 9 10
Lichtenstein AV 2018 Genetic mosaicism and cancer: cause and effect. Cancer Res. 78 1375–1378
Lin X, Kulkarni P, Bocci F, et al. 2019 Structural and dynamical order of a disordered protein: Molecular insights into conformational switching of PAGE4 at the systems level. Biomolecules 9 77
Lin X, Roy S, Jolly MK, et al. 2018 PAGE4 and conformational switching: insights from molecular dynamics simulations and implications for prostate cancer. J. Mol. Biol. 430 2422–2438
Liu L, Liu W, Wang L, et al. 2017 Hypoxia-inducible factor 1 mediates intermittent hypoxia-induced migration of human breast cancer MDA-MB-231 cells. Oncol. Lett. 14 7715–7722
Liu S, Cong Y, Wang D, et al. 2014 Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. Stem Cell Rep. 2 78–91
Liu Z, Chen M, Zhao R, et al. 2019 CAF-induced placental growth factor facilitates neangiogenesis in hepatocellular carcinoma. Acta Biochim. Biophys. Sin. 52 18–25
Louie E, Nik S, Chen J, et al. 2010 Identification of a stem-like cell population by exposing metastatic breast cancer cell lines to repetitive cycles of hypoxia and reoxygenation. Breast Cancer Res. 12 R94
Lu M, Jolly MK, Levine H, Onuchic JN and Ben-Jacob E 2013 MicroRNA-based regulation of epithelial–hybrid–mesenchymal fate determination. Proc. Natl. Acad. Sci. USA 110 18144–18149
Luzzi KJ, MacDonald IC, Schmidt EE, et al. 2017 Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am. J. Pathol. 153 865–873
Lyberopoulou A, Aravantinos G, Efstathopoulos EP, et al. 2015 Mutational analysis of circulating tumour cells from colorectal cancer patients and correlation with primary tumour tissue. PLoS One 10 e0123902
Mack SC, Witt H, Piro RM, et al. 2014 Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. Nature 506 445–450
Maffini MV, Calabro JM, Soto AM and Sonnenschein C 2005 Stromal regulation of neoplastic mammmary cells by mammary stroma. Am. J. Pathol. 167 1405–1410
Maffini MV, Soto AM, Calabro JM, Ucci AA and Sonnenschein C 2004 The stroma as a crucial target in rat mammary gland carcinogenesis. J. Cell Sci. 117 1495–1502
Mahmoudabadi G, Rajagopalan K, Getzenberg RH, et al. 2013 Intrinsically disordered proteins and conformational noise implications in cancer. Cell Cycle 12 26–31
Mani SA, Guo W, Liao M-J, et al. 2008 The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133 704–715

Mantovani F, Collavini L and Del Sal G 2019 Mutant p53 as a guardian of the cancer cell. Cell Death Differ. 26 199–212

Marine J-C, Dawson S-J and Dawson MA 2020 Non-genetic mechanisms of therapeutic resistance in cancer. Nat. Rev. Cancer 20 743–756

Meadams HH and Arkin A 1997 Stochastic mechanisms in gene expression. Proc. Natl. Acad. Sci. USA 94 814–819

McCullough KD, Coleman WB, Smith GJ and Grisham JW 1997 Age-dependent induction of hepatic tumour regression by the tissue microenvironment after transplantation of neoplastically transformed rat liver epithelial cells into the liver. Cancer Res. 57 1807–1813

McGranahan N and Swanton C 2017 Clonal heterogeneity and tumour evolution: past, present, and the future. Cell 168 613–628

Mikubo M, Inoue Y, Liu G and Tsao M-S 2021 Mechanism of drug tolerant persister cancer cells: the landscape and clinical implication for therapy. J. Thorac. Oncol. 16 1798–1809

Mintz B and Illmensee K 1975 Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc. Natl. Acad. Sci. USA 72 3585–3589

Miura H, Kondo Y, Matsuda M and Aoki K 2018 Cell-to-cell heterogeneity in p38-mediated cross-inhibition of JNK causes stochastic cell death. Cell Rep. 24 2658–2668

Mooney SM, Jolly MK, Levine H and Kulkarni P 2016 Phenotypic plasticity in prostate cancer: role of intrinsically disordered proteins. Asian J. Androl. 18 704–710

Moris N, Pina C and Arias AM 2016 Transition states and cell fate decisions in epigenetic landscapes. Nat. Rev. Genet. 17 693–703

Moyed HS and Bertrand KP 1983 hipA, a newly recognized gene of Escherichia coli K-12 that affects frequency of persistence after inhibition of murein synthesis. J. Bacteriol. 155 768–775

Nieto MA, Huang RY, Jackson RA and Thiery JP 2016 EMT: 2016. Cell 166 21–45

Nihan AK, Sugiyama N, Reddy Kalathur RK, et al. 2019 Histone deacetylases, Mbd3/NuRD, and Tet2 hydroxylase are crucial regulators of epithelial–mesenchymal plasticity and tumour metastasis. Oncogene 39 1498–1513

Niklas KJ, Dunker AK and Yruela I 2018 The evolutionary origins of cell type diversification and the role of intrinsically disordered proteins. J. Exp. Bot. 69 1437–1446

Nowell P 1976 The clonal evolution of tumour cell populations. Science 194 23–28

Ocaña OH, Córcoles R, Fabra A, et al. 2012 Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. Cancer Cell 22 709–724

Oren Y, Tsabar M, Cuoco MS, et al. 2021 Cycling cancer persister cells arise from lineages with distinct programs. Nature 596 576–582

Osorio D, Yu X, Zhong Y, et al. 2020 Single-cell expression variability implies cell function. Cells 9 14

Paek AL, Liu JC, Loewer A, Forrester WC and Lahav G 2016 Cell-to-cell variation in p53 dynamics leads to fractional killing. Cell 165 631–642

Paget S 1889 The distribution of secondary growths in cancer of the breast. Lancet 133 571–573

Pasani S, Sahoo S and Jolly MK 2021 Hybrid E/M phenotype(s) and stemness: a mechanistic connection embedded in network topology. J. Clin. Med. 10 60

Pastushenko I and Blanpain C 2019 EMT transition states during tumour progression and metastasis. Trends Cell Biol. 29 212–226

Pastushenko I, Brisebarre A, Sifrim A, et al. 2018 Identification of the tumour transition states occurring during EMT. Nature 556 463–468

Patil A, Kinoshita K and Nakamura H 2010 Hub Promiscuity in protein-protein interaction networks. Int. J. Mol. Sci. 11 1930–1943

Pearson GW 2019 Control of invasion by epithelial-mesenchymal transition programs during metastasis. J. Clin. Med. 8 646

Pillai M and Jolly MK 2021 Systems-level network modeling deciphers the master regulators of phenotypic plasticity and heterogeneity in melanoma. iScience 24 103111

Pisco AO and Huang S 2015 Non-genetic cancer cell plasticity and therapy-induced stemness in tumour relapse: ‘What does not kill me strengthens me.’ Br. J. Cancer 112 1725–1732

Prieto-Vila M, Usuba W, Takahashi R, et al. 2019 Single-cell analysis reveals a preexisting drug-resistant subpopulation in the luminal breast cancer subtype. Cancer Res. 79 4412–4425

Qin S, Jiang J, Lu Y, et al. 2020 Emerging role of tumour cell plasticity in modifying therapeutic response. Signal Transduct. Target. Ther. 5 228

Quail DF and Joyce JA 2013 Microenvironmental regulation of tumour progression and metastasis. Nat. Med. 19 1423–1437

Raj A, Peskin CS, Tranchina D, Vargas DY and Tyagi S 2006 Stochastic mRNA synthesis in mammalian cells. PLoS Biol. 4 e309

Rambow F, Rogiers A, Marin-Bejar O, et al. 2018 Toward minimal residual disease-directed therapy in melanoma. Cell 174 843–855.e59

Ramirez M, Rajaram S, Steininger R, et al. 2016 Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. Nat. Commun. 7 10690

Raser JM and O’Shea EK 2004 Control of stochasticity in eukaryotic gene expression. Science 304 1811–1814

Ravasio A, Myaing MZ, Chia S, et al. 2020 Single-cell analysis of EphA clustering phenotypes to probe cancer cell heterogeneity. Commun. Biol. 3 429
Rebecca VW and Herlyn M 2020 Nongenetic mechanisms of drug resistance in melanoma. *Annu. Rev. Cancer Biol.* **4** 315–330

Rehman SK, Haynes J, Collignon E, *et al.* 2021 Colorectal cancer cells enter a diapause-like DTP state to survive chemotherapy. *Cell* **184** 226–242.e21

Roca H, Hernandez J, Weidner S, *et al.* 2013 Transcription factors OVOL1 and OVOL2 induce the mesenchymal to epithelial transition in human cancer. *PLoS One* **8** e76773

Rossi NA, El Meouche I and Dunlop MJ 2019 Forecasting cell fate during antibiotic exposure using stochastic gene expression. *Commun. Biol.* **2** 259

Rudnick JA, Arendt LM, Klebba I, *et al.* 2011 Functional heterogeneity of breast fibroblasts is defined by a prostaglandin secretory phenotype that promotes expansion of cancer-stem like cells. *PLoS One* **6** e24605

Ruscetti M, Dadashian EL, Guo W, *et al.* 2016 HDAC inhibition impedes epithelial-mesenchymal plasticity and suppresses metastatic, castration-resistant prostate cancer. *Oncogene* **35** 3781–3795

Sacchetti A, Teeuwsen M, Verhagen M, *et al.* 2021 Phenotypic plasticity underlies local invasion and distant metastasis in colon cancer. *eLife* **10** e61461

Sahoo S, Mishra A, Kaur H, *et al.* 2021a A mechanistic model captures the emergence and implications of non-genetic heterogeneity and reversible drug resistance in ER+ breast cancer cells. *NATR Cancer* **3** zcab027

Sahoo S, Nayak SP, Hari K, *et al.* 2021b Immunosuppressive traits of the hybrid epithelial/mesenchymal phenotype. *Front. Immunol.* **12** 1664–3224

Sahoo S, Singh D, Chakraborty P and Jolly MK 2020 Emergent properties of the HNF4α-PPARγ network may drive consequent phenotypic plasticity in NAFLD. *J. Clin. Med.* **9** 870

Salgia R and Kulkarni P 2018 The genetic/non-genetic duality of drug “resistance” in cancer. *Trends Cancer* **4** 110–118

Sarkar S, Sinha SK, Levine H, Jolly MK and Dutta PS 2019 Anticipating critical transitions in epithelial-hybrid-mesenchymal cell-fate determination. *Proc. Natl. Acad. Sci. USA* **116** 26343–26352

Sasagawa Y, Nikaido I, Hayashi T, *et al.* 2013 Quartz-Seq: A highly reproducible and sensitive single-cell RNA sequencing method, reveals nongenetic gene-expression heterogeneity. *Genome Biol.* **14** 3097

Saxena K and Jolly MK 2019 Acute vs. Chronic vs. cyclic hypoxia: Their differential dynamics, molecular mechanisms, and effects on tumour progression. *Biomolecules* **9** 339

Saxena K, Sririkrishnan S, Celia-Terrassa T and Jolly MK 2020 OVOL1/2: Drivers of epithelial differentiation in development, disease, and reprogramming. *Cells Tissues Organs* **211** 183–192

Schliekelman MJ, Taguchi A, Zhu J, *et al.* 2015 Molecular portraits of epithelial, mesenchymal, and hybrid states in lung adenocarcinoma and their relevance to survival. *Cancer Res.* **75** 1789–1800

Serresi M, Kertalli S, Li L, Schnitt MJ, Dramaretska Y, Wierix J, Hulsman D and Gargiulo G 2021 Functional antagonism of chromatin modulators regulates epithelial-mesenchymal transition. *Sci. Adv.* **7** eabd7974

Sha Y, Wang S, Zhou P and Nie Q 2020 Inference and multiscale model of epithelial-to-mesenchymal transition via single-cell transcriptomic data. *Nucleic Acids Res.* **48** 9505–9520

Shachaf CM and Felscher DW 2005 Rehabilitation of cancer through oncogene inactivation. *Trends Mol. Med.* **11** 316–321

Shachaf CM, Kopelman AM, Arvanitis C, *et al.* 2004 MYC Inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature* **431** 1112–1117

Shaffer SM, Dunagin MC, Torborg SR, *et al.* 2017 Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. *Nature* **546** 431–435

Shafran JS, Jafari N, Casey AN, Györfy B and Denis GV 2021 BRD4 regulates key transcription factors that drive epithelial–mesenchymal transition in castration-resistant prostate cancer. *Prostate Cancer Prostatic Dis.* **24** 268–277

Sharma A, Merritt E, Hu X, *et al.* 2019 Non-genetic intratumour heterogeneity is a major predictor of phenotypic heterogeneity and ongoing evolutionary dynamics in lung tumours. *Cell Rep.* **29** 2164–2174

Sharma SV, Lee DY, Li B, *et al.* 2010 A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141** 69–80

Shen S, Faouzi S, Souquere S, *et al.* 2020a Melanoma persister cells are tolerant to BRAF/MEK inhibitors via ACOX1-mediated fatty acid oxidation. *Cell Rep.* **33** 108421

Shen S, Vagner S and Robert C 2020b Persistent cancer cells: the deadly survivors. *Cell* **183** 860–874

Shibue T and Weinberg RA 2017 EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat. Rev. Clin. Oncol.* **14** 611–629

Shyakhitina Y, Moran KL and Portal MM 2021 Functional and alternate sources of gene-expression variability. *Nature* **595** 198–201

Singh A, Razooky BS, Dar RD and Weinberger LS 2012 Dynamics of protein noise can distinguish between metastatic cancer cells enter a diapause-like DTP state to survive chemotherapy. *Cell* **184** 226–242.e21

Srivastava BK, beam AN, Tice PA, *et al.* 2021 Single-cell lineage and transcriptome reconstruction of metastatic cancer cells: the deadly survivors. *Cell* **183** 860–874

Simeonov KP, Byrns CN, Clark ML, *et al.* 2021 Single-cell lineage and transcriptome reconstruction of metastatic cancer reveals selection of aggressive hybrid EMT states. *Cancer Cell* **39** 1150–1162

Singh A, Razooky BS, Dar RD and Weinberger LS 2012 Dynamics of protein noise can distinguish between alternate sources of gene-expression variability. *Mol. Syst. Biol.* **8** 607
Singh D, Bocci F, Kulkarni P and Jolly MK 2021 Coupled feedback loops involving PAGE4, EMT and Notch signaling can give rise to non-genetic heterogeneity in prostate cancer cells. *Entropy* **26** 288

Sonnencchein C and Soto AM 2015 Cancer metastases: So close and so far. *J. Natl. Cancer Inst.* **107** 17–20

Sonnencchein C, Soto AM, Rangarajan A and Kulkarni et al. 2010 Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* **459** 428–432

Stably O’Shea C, Willemoës M, Theisen F, Kragelund BB and Skriver K 2017 Eukaryotic transcription factors: Paradigms of protein intrinsic disorder. *Biochem. J.* **474** 2509–2532

Steinway SN, Zaﬁnodo JGT, Michel PJ, et al. 2015 Combinatorial interventions inhibit TGFβ-driven epithelial-to-mesenchymal transition and support hybrid cellular phenotypes. *NPJ Syst. Biol. Appl.* **1** 15014

Stamatoiu N, Lehman ML, Wang C, et al. 2019 Molecular portrait of epithelial–mesenchymal plasticity in prostate cancer associated with clinical outcome. *Oncogene* **38** 913–934

Su Y, Bintz M, Yang Y, et al. 2019 Phenotypic heterogeneity and evolution of melanoma cells associated with targeted therapy resistance. *PLoS Comput. Biol.* **15** e1007034

Su Y, Wei W, Robert L, et al. 2017 Single-cell analysis resolves the cell state transition and signaling dynamics associated with melanoma drug-induced resistance. *Proc. Natl. Acad. Sci. USA* **114** 13679–13684

Subbalakshmi AR, Kundnani D, Biswas K, et al. 2020 NFATc acts as a non-canonical phenotypic stability factor for a hybrid epithelial/mesenchymal phenotype. *Front. Oncol.* **10** 1794

Süel GM, Garcia-Ojalvo J, Liberman L and Elowitz MB 2006 An excitable gene regulatory circuit induces transient cellular differentiation. *Nature* **440** 545–550

Tan TZ, Miow QH, Miki Y, et al. 2014 Epithelial-mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. *EMBO Mol. Med.* **6** 1279–1293

Tang DG 2012 Understanding cancer stem cell heterogeneity and plasticity. *Cell Res.* **22** 457–472

Tantale K, Mueller F, Kozulic-Pirher A, et al. 2016 A single-molecule view of transcription reveals convoys of RNA polymerases and multi-scale bursting. *Nat. Commun.* **7** 12248

Tarin D 2011 Cell and tissue interactions in carcinogenesis and metastasis and their clinical significance. *Semin. Cancer Biol.* **21** 72–82

Thankamony AP, Saxena K, Murali R, Jolly MK and Nair R 2020 Cancer stem cell plasticity—a deadly deal. *Front. Mol. Biosci.* **7** 79

Tian X-J, Zhang H and Xing J 2013 Coupled reversible and irreversible bistable switches underlying TGFβ-induced epithelial to mesenchymal transition. *Biophys. J.* **105** 1079–1089

Tièche CC, Gao Y, Bührer ED, et al. 2018 Tumour initiation capacity and therapy resistance are differential features of emt-related subpopulations in the NSCLC cell line A549. *Neoplasia* **21** 185–196

Tirosh I, Izar B, Prakadan SM, et al. 2016 Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **352** 189–196

Toneff MJ, Sreekumar A, Tinnirello A, et al. 2016 The Z-cad dual fluorescent sensor detects dynamic changes between the epithelial and mesenchymal cellular states. *BMC Biol.* **14** 47

Tripathi S, Levine H and Jolly MK 2020 The physics of cellular decision-making during epithelial-mesenchymal transition. *Annu. Rev. Biophys.* **49** 1–18

Tripathi SC, Peters HL, Taguchi A, et al. 2016 Immuno-proteasome deficiency is a feature of non-small cell lung cancer with a mesenchymal phenotype and is associated with a poor outcome. *Proc. Natl. Acad. Sci. USA* **113** E1555–E1564

Tsafou K, Tiwari PB, Forman-Kay JD, Metallo SJ and Toretsky JA 2018 Targeting intrinsically disordered transcription factors: Changing the paradigm. *J. Mol. Biol.* **430** 2321–2341

Tyler AL, Asselbergs FW, Williams SM and Moore JH 2009 Shadows of complexity: what biological networks reveal about epistasis and pleiotropy. *BioEssays* **31** 220–227

Udyavar AA, Wooten DJ, Hoeksma M, et al. 2017 Novel Hybrid phenotype revealed in small cell lung cancer by a transcription factor network model that can explain tumour heterogeneity. *Cancer Res.* **77** 1063–1074

Urban EA and Johnston RJ 2018 Buffering and amplifying transcriptional noise during cell fate specification. *Front. Genet.* **9** 591

van Boxtel C, van Heerden JH, Nordholt N, Schmidt P and Bruggeman FJ 2017 Taking chances and making mistakes: non-genetic phenotypic heterogeneity and its consequences for surviving in dynamic environments. *J. R. Soc. Interface* **14** 20170141

Veening J, Smits WK and Kuipers OP 2008a Bistability, epigenetics, and bet-hedging in bacteria. *Annu. Rev. Microbiol.* **62** 193–212

Veening JW, Stewart EJ, Berngruber TW, et al. 2008b Bet-hedging and epigenetic inheritance in bacterial cell development. *Proc. Natl. Acad. Sci. USA* **105** 4393–4398

Vega S, Morales AV, Ocaña OH, et al. 2004 Snail blocks the cell cycle and confers resistance to cell death. *Genes Dev.* **18** 1131–1143
Vander Velde R, Yoon N, Marusyk V, et al. 2020 Resistance to ALK targeting therapies as a gradual Darwinian adaptation to inhibitor specific selective pressures. *Nat. Commun.* 11 2393

Waddington CH 1957 A discussion of some aspects of theoretical biology; in *The strategy of the genes* (London: Routledge)

Wang J, Zhang K, Xu L and Wang E 2011 Quantifying the Waddington landscape and biological paths for development and differentiation. *Proc. Natl. Acad. Sci. USA* 108 8257–8262

Wang N, Zheng J, Chen Z, et al. 2019 Single-cell microRNA-mRNA co-sequencing reveals non-genetic heterogeneity and mechanisms of microRNA regulation. *Nat. Commun.* 10 95

Watanabe K, Panchy N, Noguchi S, Suzuki H and Hong T 2019 Combinatorial perturbation analysis reveals divergent regulations of mesenchymal genes during epithelial-to-mesenchymal transition. *NPJ Syst. Biol. Appl.* 5 21

Welch DR and Hurst DR 2019 Defining the hallmarks of metastasis. *Cancer Res.* 79 3011–3027

Wooten DJ and Quaranta V 2017 Mathematical models of cell phenotype regulation and reprogramming: Make cancer cells sensitive again! *BBA Rev. Cancer* 1867 167–175

Xie M, Lu C, Wang J, et al. 2014 Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med.* 20 1472–1478

Yang J, Mani SA, Donaher JL, et al. 2004 Twist, a master regulator of Morphogenesis, plays an essential role in tumour metastasis. *Cell* 117 927–939

Yeo SK and Guan JL 2017 Breast cancer: multiple subtypes within a tumour? *Trends Cancer* 3 753–760

Youssoufian H and Pyeritz RE 2002 Mechanisms and consequences of somatic mosaicism in humans. *Nat. Rev. Genet.* 3 748–758

Yu M, Bardia A, Wittner BS, et al. 2013 Circulating breast tumour cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* 339 580–584

Zhang Z and Tjian R 2018 Measuring dynamics of eukaryotic transcription initiation: challenges, insights and opportunities. *Transcription* 9 159–165

Zhang J, Tian X-J, Zhang H, et al. 2014 TGF-β-induced epithelial-to-mesenchymal transition proceeds through stepwise activation of multiple feedback loops. *Sci. Signal.* 7 91

Zhu Y, Shi C, Zeng L, et al. 2020 High COX-2 expression in cancer-associated fibroblasts contributes to poor survival and promotes migration and invasiveness in nasopharyngeal carcinoma. *Mol. Carcinog.* 59 265–280

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