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Explaining the dynamics of tumor aggressiveness: at the crossroads between biology, artificial intelligence and complex systems

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Abstract

Facing metastasis is the most pressing challenge of cancer research. In this review, we discuss recent advances in understanding phenotypic plasticity of cancer cells, highlighting the kinetics of cancer stem cell and the role of the epithelial mesenchymal transition for metastasis. It appears that the tumor micro-environment plays a crucial role in triggering phenotypic transitions, as we illustrate discussing the challenges posed by macrophages and cancer associated fibroblasts. To disentangle the complexity of environmentally induced phenotypic transitions, there is a growing need for novel advanced algorithms as those proposed in our recent work combining single cell data analysis and numerical simulations of gene regulatory networks. We conclude discussing recent developments in artificial intelligence and its applications to personalized cancer treatment.

Keywords: , cancer stem cells, phenotypic switching, metastasis, precision medicine

Cancer stem cells, phenotypic switching and metastasis

Cancer stem cells (CSCs) have been defined as cells with the capability of self-renewing, generating an heterogeneous population within the tumor [1]. According to this view, tumors expand in a hierarchical way, with CSC at the apex of the tree. The other model of tumor growth is the stochastic one, postulating that cancer cells are heterogeneous but are all able to sustain tumor growth [2]. There was evidence in support of both hypotheses, but according to the recent literature the two models are actually not in contrast. Indeed, there is a growing support to the idea that the cancer cell population is dynamic, with cancer cells (CCs) that are able to revert into CSCs. This general phenomenon is called *phenotypic switching*.

A brief summary of the most relevant papers showing a phenotypic switching in tumor cells are discussed below. In 2011, a paper pointed out the possibility that non-CSCs breast cancer cells can revert to a stem cell like state even in the absence of mutations [3]. In melanoma, a small population of CSC-like histone demethylase 5B (JARID1B) positive cells has been shown to be

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dynamically regulated in a way that differs from the standard hierarchical CSC model [4]. Micro-environmental factors, such as transforming groth factor beta (TGFbeta), are found to enhance the rate of switch from non-CSC cells to the CSC state [5]. In this connection, ATP Binding Cassette Subfamily G Member 2 (ABCG2) negative cells isolated from human melanoma biopsies express again this marker after few passages in vitro [6]. Instead of a switch to a CSC state driven by genetic mutations, recent papers suggest a crucial role of epigenetic factors modulated by the environment in human intestine and melanoma cells [3, 7, 8, 9, 10, 11, 12, 13]. Moreover, the possibility to limit stemness by modifying the microenvironments has also been shown in a zebrafish-specific melanoma cell line (ZMEL1) [13, 14]. Our group contributed to this specific goal showing the critical role of the microenvironment on specific target of stemness such as Wingless-type integration (Wnt) and Phosphoinositide 3-kinase (PI3K)-pathways by miRNAs in human melanoma [12]. In fact, we showed that cancer cells can switch into CSCs thanks to a complex miRNA network which in turns regulates crucial pathways of stemness such as Wnt and PI3K [12]. On the other side, we demonstrated that the miRNA network is activated when the CSC population is reduced below a threshold, implying that cancer cells are sensitive to the number of CSCs in the bulk [12]. This is a typical feature of normal stem cells which have to maintain their number exactly constant due to space limitations in the niche. Recently in stem cells, the spatial-dependent activation of Wnt signaling was shown to dictate differentiation and spatial confinement in the niche [15] and to regulate the number of stem cells [16].

Based on those studies, phenotypic switching emerges as a tightly regulated process that is dependent on the numbers of CSCs in the bulk, implying the presence of a crosstalk between the cells. Taken together, this evidence clearly shows that a scenario based only on a stochastic process is too simple for tumor development. Cancer cells show a high degree of plasticity and there is an equilibrium between CSCs and their more differentiated progeny. One of the most important consequences of phenotypic switching is related to metastasis. The metastatic process is usually interpreted based on the idea that each metastasis arises from the clonal growth of a single tumor cell that has detached from tumor mass and migrated elsewhere, however the recent evidence showed that this view has to be revised [17]. For example the circulating tumor cells (CTCs) have been found to move as clusters of cells in the blood of patients [18]. Therefore the classical view is that of a continuous biological process consisting in a sequence of distinct steps: from local invasion to intravasation, from extravasation to colonization. According to this view, it is critical to understand the complex array of biological factors determining the progression from primary malignancy to overt metastasis. When and why a tumor becomes able to relapse is, however, still unknown. According to the phenotypic switching view, migration to a distant site could be done by CSCs but also by CCs which could then switch and seed new metastasis by undergoing an epithelial-mesenchymal transition (EMT). To switch the phenotype, the environment might play a critical role as discussed recently [17]. Moreover, according to the literature, CSCs migration appears unlikely since they typically represent a small fraction of the cancer cell population. In light of this evidence, instead of targeting CCs or CSCs, it could be more promising to target the niche to overcome cellular resistance and prevent metastasis (see Fig. 1).

Plasticity in cancer cells: the epithelial mesenchymal transition

The EMT, controlled by a numbers of transcription factors [19], is associated with the loss of cell-cell adhesion and the gain of invasive traits and is therefore a hallmark of plasticity within

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a stem cell population and appears particularly relevant for tumors. Almost 80% of human malignancies which origin from epithelial tissues express mesenchymal markers, being usually associated with a more aggressive phenotype [19, 20, 21, 22]. More recently, interesting evidence shows that the EMT is a multiple process where cells express a mix of markers, characteristic of both epithelial and mesenchymal cells [23, 24, 25]. Hence, the rigid distinction between epithelial and mesenchymal phenotype is becoming more fuzzy: Cancer cells can acquire hybrid phenotypes, combining invasive capabilities with intracellular adhesion [24, 25]. It turns out that hybrid cells are extremely aggressive and associated to a poor patient outcome [26, 27].

In a recent paper, we have investigated this aspect using a combination of numerical simulations of a Boolean network model of the EMT pathways and the analysis of bulk and single cell gene expression data [28]. Interestingly, we showed that the EMT involves the transit through a multitude of meta-stable states, corresponding to highly aggressive hybrid cells that can easily switch under external and internal perturbations [28]. Our study allowed to reconstruct the topography of the phenotypic landscape, as originally envisaged in general terms by Waddington more than sixty years ago [29] and rationalized as the possible attractors of the relevant gene regulatory network [30, 31, 32, 33, 34, 35, 36, 37] In contrast to the smooth landscape depicted by Waddington, the landscape emerging from the kinetics of the EMT regulatory network is extremely complex, with a hierarchically organized set of valleys and mountains corresponding to an impressive number of cell phenotypes separating epithelial and mesenchymal states (Fig. 2) [28]. Hence, phenotypic switching can take multiple paths and produce a variety of outcomes corresponding to the astonishing the complexity of a cancer cell population. Furthermore, the large number of states expressed by the network confirms that we should abandon the rigid distinction between epithelial or mesenchymal cells. We should think instead about a continuum of possible cell phenotypes with varying degrees of plasticity. In light of these findings, metastasis could be due to one of these CCs with hybrid phenotype that can easily migrate and switch in the right environment. According to this complex landscape, Wicha's group showed that CSCs mantain plasticity to transition between mesenchymal-like and epithelial-like stated in response to the tumor microenvironment [38]. There are also consequences for the development of new therapeutic strategies based on the possibility to target essential mechanisms controlling the EMT through the microenvironment [39].

Another interesting aspect that might be linked to EMT is the presence of dormant tumor cells. It has been shown in the last decade that disseminated cancer cells might display slow growth to adapt to the host microenvironment for the metastatic expansion [40, 41, 42, 43, 44]. There is a tropism specific to each organ that can help the metastatic process and the expansion of the tumor [40, 41, 42, 43, 44]. CTCs or disseminated tumor cells (DTCs) can stay dormant through inhibition of cell proliferation and activation of cell survival pathways [45, 46]. These cells are undetectable for long period and might be the reason for the relapse of the tumor [47, 48, 49, 50]. It is remarkable that DTCs have also been observed in primary tumors undergoing EMT to develop migratory and invasive phenotypes [50, 51].

As shown in Fig. 3, in an epithelial primary tumor, some cancer cells, either CCs or CSCs, could switch to a mesenchymal phenotype, enter the nearest vessel, reach a secondary site through the circulation, extravasate and become dormant. When the environment is permissive, these cells or part of them could switch back to an epithelial phenotype and grow (Fig.3). The strict relationship between the tumor cells and the microenvironment seems to be a critical factor for the reactivation of dormant metastatic cells. The transition to the mesenchymal phenotype is important for the formation of micrometastasis. The presence therefore of a favourable microenvironment is an essential condition during metastasis development. Further-

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more, metastatic niche formation needs the involvement of inflammation, immunosuppression, angiogenesis/vascular permeability, organotropism, lymphoangiogenesis and cellular reprogramming [52]. In this connection, macrophages have been shown to be critical, in particular a subset of macrophages (Tumor associated macrophages) [53, 54, 55, 56]. The reactivation of the dormant cells appears to involve many factors including TGFbeta that support the production of periostin from stromal fibroblasts and endothelial cells in the vascular area [57, 58]. Finally, the possibility to neutralize dormant cells acting through the niche can be an important support to chemotherapy or help maintain a dormant state.

Cancer niche: new challenges

Tumor cells require nutrients and signals from the surrounding microenvironment which also regulates a dynamic balance between CSCs and CCs. The tumor niche comprises different types of cells in addition to a non-cellular component (cytokines, growth factors etc), mainly cancer-associated fibroblasts (CAF) and macrophages. Macrophages are present in the tumor microenvironment together with immune cells, endothelial cells, fibroblasts and mesenchymal stromal/stem cells and communicate with the tumor. They can drive different states of activation in response to different microenvironment: M1 (the killing phenotype) and M2 (the healing phenotype). In fact, while M1 was shown to help a pro-inflammatory environment, M2 is an anti-inflammatory one. In recent years, however, many papers showed that macrophages polarize in a more broad spectrum of phenotypes involved in various immunoregulatory disorders. Macrophages play an important role for the control and clearance of infections, removal of debrits and dead cells, promoting tissue repair and would healing. However they can also be involved in inflammation and tissue damage helping the tumor to grow and disseminate. In particular, M2 cells help the tumor while M1 exhert an anti-tumor activity [59]. These cells are incredibly plastic and can switch from one phenotype to the other [60, 61]. Polarization of macrophages to a specific phenotype is due to local cytokines [61].

According to the literature, tumor associated macrophages (TAMs) are the major infiltrating leukocytes of the tumor micro-environment [62]. They have usually a M2-like phenotype and an immunosuppressive activity, producing growth factors for the tumor cells [62]. TAMs also promote dissemination of tumor cells enhancing angiogenesis and matrix degradation, suppressing Th1 immne ativity [63]. In particular, the production of matrix metalloproteases (MMP), osteonectin and cathepsins by TAMs helps tumor cells to escape from the original mass and invade the surrounding tissue. In general, there is ample evidence in breast and ovarian cancer showing that TAM infiltration is correlated to a poor patient survival [63]. On the other hand, direct targeting of TAMs or re-polirization toward M1 phenotype in the microenvironment seems to represent an attractive way to affect the tumor development towards a more aggressive phenotype. Interestingly, recent studies showed that TAMs are able to orchestrate EMT [64] in pancreatic cells. Furthermore, TAMs promote CSC-like properties via TGF-beta1, inducing EMT in hepatocellular carcinoma [65]. These studies and others suggest that TAMs play a critical role in the development of an environment that helps the premetastatic niche, by reteining circulating cells and helping their growth [54]. There is evidence in the literature suggesting that TAMs are associated with CSCs [66, 67]. All together, these data strongly support the idea to target TAMs in order to destabilize the tumor microenvironment [68].

Cancer-associated fibroblasts (CAF) are major components of the stroma of tumors. Fibroblasts can show an altered phenotype characterized by an increased expression of markers such

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as alpha-smooth muscle actin (a-SMA) and fibroblast activation protein (FAP) and also an increased expression of extracellular matrix proteins such as collagen type I [69]. Both migration and invasion are supported by CAF-secreted factors (i.e TGFbeta) helping the transition from epithelial to mesenchymal phenotype of the tumor [70]. CAFs are shown to play a critical role in the organization of the niche which helps tumor to invade. In fact, considering that one of the most important function of the fibroblasts is to deposit and assembly the extracellular matrix, CAFs exhibit an abnormal activity in terms of extracellular matrix regulation, releasing high levels of fibronectin and type I collagen as well as high level of expression and activation of MMPs [71]. The effect of CAFs is either on the tumor cells and on the stroma. CAFs were also shown to modulate the tumor vasculature, a critical element to refurbish the tumor cells with nutrients and oxygen and removing waste products. Due to the excessive production of these factors, the vessels are abnormal with structural instability due to defective coverage with pericytes and basement membrane [72]. Therefore CSFs can modulate the vasculature through the release of for example VEGF, CXCL12 and MMPs, they can modulate the immune systems (TGFbeta), the extracellular matrix and the same cancer cells. It is thereby quite clear that the possibility to target these cells in the tumor niche could destabilize the tumor, help maintaining it a quiescent state or lead cells to death. Hypoxia is a typical feature of tumors and has been shown to control the remodelling of extracellular matrix [73] but also affect CAFs [74].

Targeting the niche where tumor cells live seem to be promising because of the heterogeneity of the tumor population and the difficulty to target them. CAFs and TAMs seem to be particular relevant for the development of the tumor toward a more aggressive phenotype.

Artificial intelligence, personalized medicine and real world data

Two great revolutions happened in recent years: the possibility to store a great amount of data and the growing speed of computers which are able to perform more operations in a shorter time. These two simple technological improvements are already able to speed up many human daily functions and also have an impact on medicine, including cancer. Computer algorithms and data analysis tools are behind several consumer services from travel agencies to recommendation systems for books, movies and music. There is an industrial revolution that started first in the society and in the relationship between people (driven by commonly used social media) and has finally reached medicine, where it is changing our approach to understand and fight diseases. Data sets grow rapidly in part because they are increasingly gathered by cheap and numerous information-sensing internet devices such as mobile phones. These technologies are entering in medicine too, speeding up the collection and the amount of data. For instance, epidemic surveillance can now rely on large but low quality data from social media to complement traditional public health approaches. Algorithms for automatic image analysis are rapidly outperforming trained humans in the classification of certain types of cancers [75].

The classical biological approach to study cancer is based on experiments carried out in a wet laboratory, sharing of the results with the scientific community through scientific publications and finally dissemination at conferences and seminars. Throughout this process, the research community is storing a huge amount of biological data (from genomes and transcriptomes to bioptic samples) related to tumors. Thanks to recent improvements in computer power and algorithmic performance, we can try to extract useful information from all these data. It is well known that artificial intelligence (AI) works very well on image recognition and indeed this has been its first application on biology. As mentioned above, skin cancer has been recently classified using AI, showing excellent results when compared with traditional analysis [75]. In

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another recent paper, the authors used deep learning based tissue analysis to predict the outcome for colorectal cancer [76] . Other possible applications are, however, still under investigation and require additional effort. One important example is the idea to connect genomic data to the disease phenotype through a maching learning or a deep learning approach. Earlier approaches to predict clinical outcome in cancer patients using machine learning on selected gene expression data have been shown, however, to suffer from serious shortcomings [77]. The main reason is that the number of available samples was too small to draw statistically firm and biologically meaningful conclusions from the data [77].

Hence, the most pressing issue in the field is to develop computational tools that are able to integrate and analyze data coming from different sources and, at the same time and more importantly, to ask the right questions to the data. Our group started about ten years ago an interdisciplinary program combining wet laboratory experiments with computational approaches and tools stemming from the physics of complex systems (for a comprehensive review see [78]). At that time, few biologists understood the great power of sharing expertise between medicine, mathematics and physics. Now we have developed powerful tools which pave the way to the development of a personalized medicine based on artificial intelligence [17] . Combining data into large sets allowed us to better explore hidden but relevant information that would not appear when analyzing small data sets because of the inevitable background noise. As discussed in a recent article [79], big data analysis in biology is difficult and one of the main sources of difficulty is related to the fact that data shared within the scientific community are not uniform in their format. Our group used an artificial intelligence approach to address the question of the search for common factors shared in obesity and breast cancer, motivated by the high frequency of obese females developing breast cancer [80]. To reach this goal, the possibility to integrate all the data available in the literature was crucial, since each study usually reports results from just few cases. We need a large number of cases to break the *curse of dimensionality*, inevitable in the search for a signature among 20000 genes using a 20 samples. We solved the problem by algorithms combining singular value decomposition and pathways deregulation analysis [80]. In this way, we identified a signature of 38 highly significant genes that are differentially expressed in obese subjects and a set of pathways that are shared between obesity and breast cancer. Finally, we showed that this gene expression signature is induced by obesity rather than emerging from a genetic fingerprint, by analyzing a large cohort of monozygotic twins [81].

Pharmaceutical companies are also changing their approach and try to develop new drugs using machine learning [82] but they are also starting to appreciate the potentially interesting information hidden in already available data. Real-world data (RWD), in the definition of the International Society for Pharmacoeconomics and Outcome Research, include all the data going beyond what is usually collected in phase III clinical trials and also any outcome that is not purely interventional [83]. Many innovative aspects are included in RWD. First, health-care decision makers are now starting to devise policies based on integrated evidence coming from a multitude of sources. This is important because it can provide information that goes beyond what has been obtained in the trial, such as the way how a particular drug works in populations not covered by the trial [84]. Secondly, RWD include information about the actual treatment patients received, also including co-morbidity, so that RWD can be used to study the effect of multiple interventions. The main problem now is the availability of good quality RWD and sufficiently representative databases, since in many countries databases are incomplete.

The possibility to devise a personalized medicine could be achieved, in our opinion, by the convergence between the development of new algorithmic strategies to investigate already available biological data in genomics, transcriptomics and images combined with the acquisition of

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new information through RWD. Beside algorithms needed to extract and visualize information from data, we also need predictive models to estimate the probability of therapeutic success of each strategy.

Conclusions: Precision medicine and tumor plasticity

The idea that tumors are highly heterogeneous is already mainstream in the field of cancer biology. The plasticity of tumor cells driven by the microenvironment, that under the right conditions induces cells to switch and seed new metastasis, is becoming an additional hallmark of cancer. Disentangling the evolution of single cells inside the tumor opens a new interesting area to understand better the complex relationship between tumor cells and the environment. Our new understanding of tumor plasticity and the important role of the microenvironment in controlling this process provides concrete answers to the quest for precision medicine [28]. To this end, we need to combine innovative algorithms for single cells gene expression analysis with predictive computational models driving the therapeutic intervention. We have discussed in depth the important role played by the microenvironment to affect the state of tumor cells (EMT/MET), therefore one promising aspect is to target of the microenvironmental instead of the tumor cells to control their plasticity.

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Figure captions

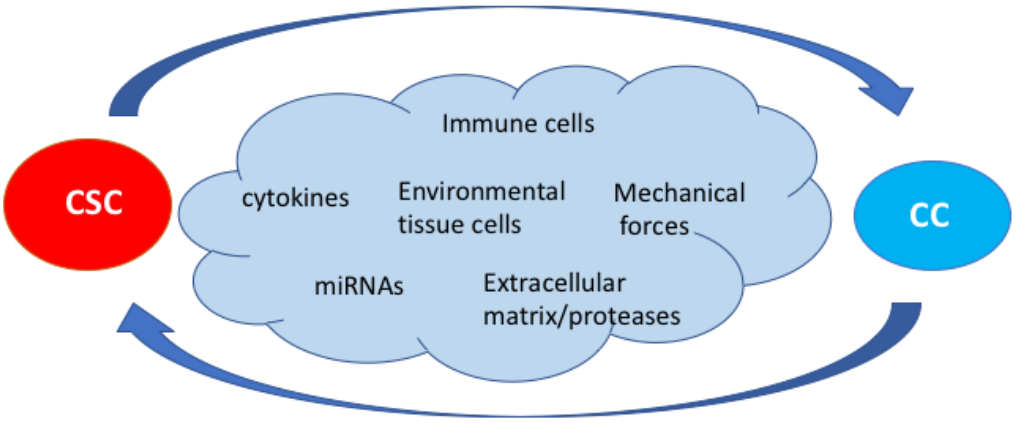

Figure 1: **Phenotypic switching of CSCs.** Phenotypic transitions between CCs and CSCs are controlled by a large cohort of environmental factors..

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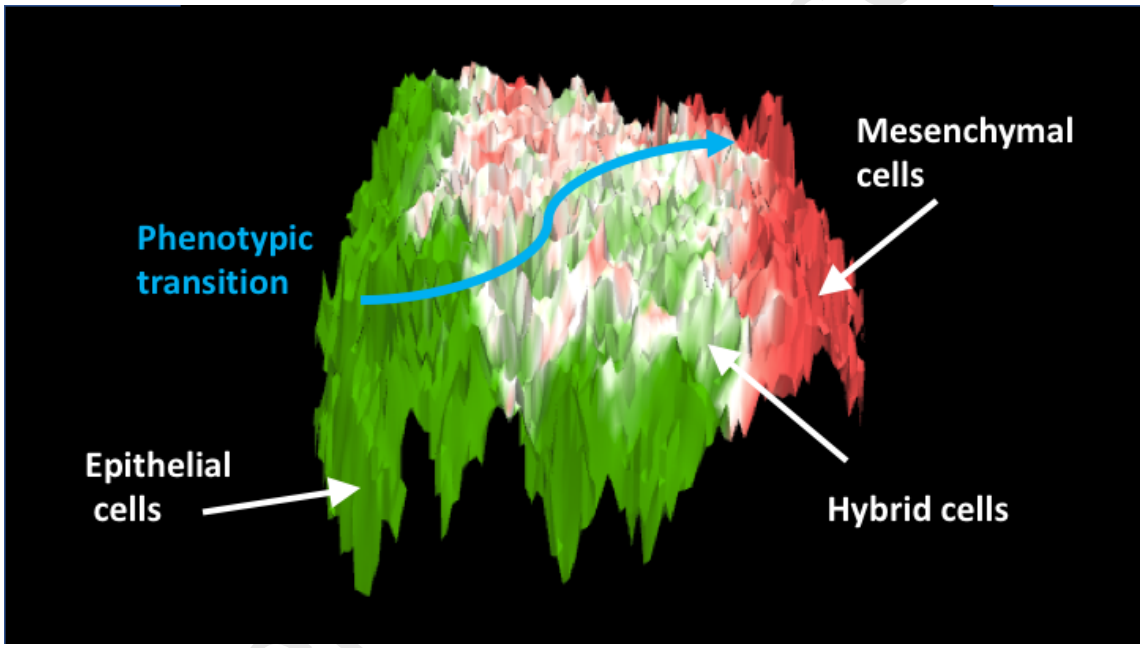

Figure 2: **Topography of the epithelial mesenchymal transitions.** Epithelial and mesenchymal phenotypes are arranged in a complex hierarchical landscape with multiple possible transition. A multitude of hybrid states reside at the top of the landscape and owing to their reduced stability can rapidly change into a different phenotype.

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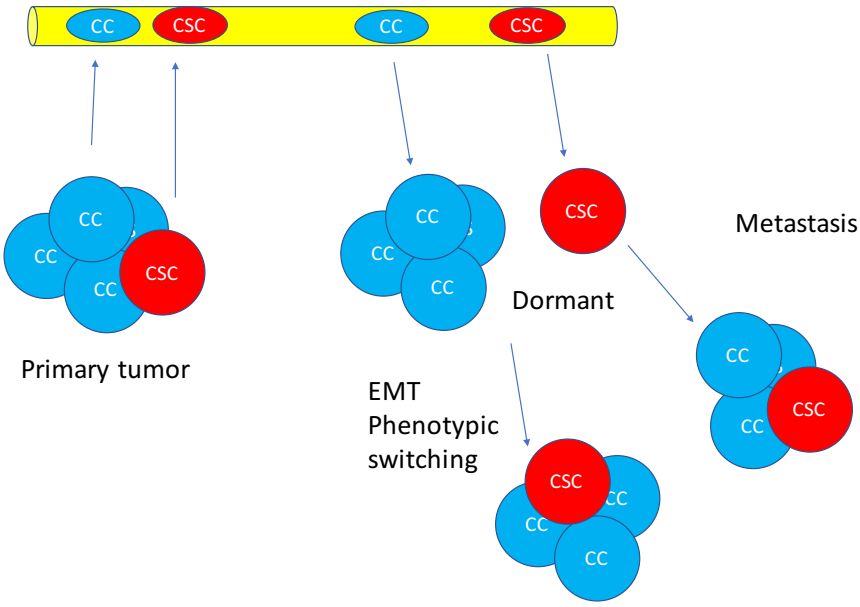

Figure 3: **Phenotypic transitions and metastasis.** Epithelial cancer cells, either CCs or CSCs, can acquire a mesenchymal phenotype, intravasate, then extravasate at a distant site and become dormant. Under favorable conditions, dormant cells can switch back to the epithelial state and grow.