Current Insights into Clinical Dormancy and Metastasis

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Abstract

Survival after breast cancer diagnosis and treatment has improved markedly, however recurrence is still a treatment challenge for clinicians. The underlying cause for late recurrence after a long disease-free period is still unknown. It is known that undetectable metastases exist in two states, with different mechanisms playing a role in each dormancy state. In tumour mass dormancy, extrinsic factors such as angiogenesis and immune surveillance are in equilibrium with the tumour cells to maintain dormancy. In tumour cell dormancy, mechanisms intrinsic to the isolated tumour cells can dictate a dormant cellular state. The process of senescence, or pathways leading to cell cycle arrest, may be the key to unlocking the mystery behind these tumour cell deposits. With greater knowledge of the mechanisms that cells can dictate a dormant cellular state. The process of senescence, or pathways leading to cell cycle arrest, may be the key to unlocking the mystery behind these tumour cell deposits. With greater knowledge of the mechanisms that extrinsic factors such as angiogenesis and immune surveillance are in equilibrium with the tumour cells to maintain dormancy. In tumour cell dormancy, mechanisms intrinsic to the isolated tumour cells can dictate a dormant cellular state. The process of senescence, or pathways leading to cell cycle arrest, may be the key to unlocking the mystery behind these tumour cell deposits. With greater knowledge of the mechanisms that control undetectable disseminated disease, we have the opportunity to target these pathways to enable therapeutic strategies against metastatic disease.

Survival after a cancer diagnosis has improved markedly with the advancement of modern therapies, leading to a paradigm shift in cancer epidemiology towards chronic disease. With greater longevity, there is a new challenge in treating patients with metastatic recurrence long after successful treatment of the primary tumour.
The disease-free period leading to recurrence, long after the expected timeframe, is defined as clinical dormancy and is a feature of several types of cancers, including follicular thyroid cancer, prostate cancer, B-cell lymphoma, melanoma, and breast cancer. While there has been research exploring the mechanisms of primary tumour dormancy, the aim of this review is to summarise what is known about metastatic dormancy.

Metastases are a significant cause of patient demise and are still a treatment challenge for clinicians. Current therapies are not very effective in treating recurrent disease, and technologies in use today limit our evaluation of interval disease. With increasing evidence to support the occurrence of metastasis as an early event in tumourigenesis, there has been a focus on early detection and monitoring and in particular, on isolating and characterising circulating tumour cells (CTCs) and disseminated tumour cells (DTCs). While many cells may be released from the primary tumour from early stage disease onwards, only a very low percentage will form macrometastases. The presence of these cells can be misleading as a prognostic indicator, and can potentially result in overtreatment of the patient. Nonetheless, CTC and DTC may provide the pool from which clinically dormant disease progresses to advanced disease, and further characterisation of the biological pathways involved with protracted metastasis and the metastatic potential of these cells is therefore imperative for our understanding of clinical dormancy and for the development of more effective therapies.

Figure 1: A theoretical model for the mechanisms involved in dormancy.

**Tumour mass dormancy**

Tumour mass dormancy refers to a state in which a clinically undetectable tumour mass is held in homeostasis by external mechanisms. Although we cannot directly detect tumour mass dormancy in patients, we can predict its existence from the presence of CTCs long after the treatment and removal of the primary tumour. Current limitations of our understanding of CTCs are due in part to suboptimal analytical tools, often capturing only a small fraction of the CTC population when using capture antibodies that bind to EpCAM, HER2 and/or EGFR. Another limitation is our poor understanding of the biology of these micrometastases and thus the unmet promise of clinical utility. To date, only numbers of CTCs greater than 5 per 7.5 ml blood in breast cancer are predictive of outcome and response to therapy, indicating significant clinical and biological heterogeneity in the majority of patients with low to moderate numbers of CTCs.

The proposed mechanisms behind tumour mass dormancy are well described. Once tumour cells grow beyond a size that tissue vasculature can support, neovascularisation is required to enable growth. Limitations in factors involved in angiogenesis prevent the micrometastasis from expanding. A successful activation of angiogenesis, known as the angiogenic switch, contributes to the expansion of the micrometastasis into a macrometastasis. Immunosurveillance is another mechanism that keeps the overall tumour burden in check, by removing tumour cells that induce an immune response. In addition, the micrometastasis is in equilibrium between cell death and cell renewal, the end result being a dynamic but dormant tumour mass. Any disruption in this delicate balance can favour a growth phase, with subsequent development of a macrometastasis.

**Tumour cell dormancy**

Pre-clinical modelling of tumour cell dormancy has been challenging due to limitations in current technology and to a lack of highly specific tumour cell dormancy biomarkers. In vivo, dormant tumour cells have been observed long after treatment of the initial primary tumour. As yet, we are still uncertain about the molecular events that result in their presence in tissues, their sustained viability over many years and the mechanisms by which they maintain their clinical indolence. What we do know is that current chemotherapeutic treatments are not effective at killing these cells. It is imperative therefore, to understand the pathways driving dormancy, to enable us to manipulate these pathways for future targeted therapy.

As outlined below, DTCs may develop mechanisms to allow them to survive for long periods in tissues such as bone marrow. Though we are unable to study DTCs...
directly, it is predicted that their stem cell properties and their transition from an epithelial state to a mesenchymal state, known as epithelial to mesenchymal transition, may explain their continued existence. However, it is not only their cellular properties that determine the dormant phenotype in DTCs. There is a dynamic equilibrium between the microenvironment and intracellular and genetic processes of the tumour cells that give rise to the prolonged viability of these dormant cells.

The epithelial to mesenchymal transition has been widely accepted as a model to describe the escape of tumour cells from the primary mass and is predicated on the ability of individual cells to reversibly change their phenotype from epithelial to mesenchymal. CTCs are evidence of this theory, as these cells can exhibit both epithelial and mesenchymal markers. Moreover, the reverse process, mesenchymal to epithelial transition, is required to enable proliferation at the secondary site. While it is not known what may trigger DTCs to revert to the proliferative epithelial cell state, targeting these mechanisms may provide a therapeutic avenue.

Cancer stem cells have multiple properties that may be applicable to DTCs and their ability to survive in vivo. They are slow cycling cells, they are immuno-evasive and they are resistant to conventional chemotherapy and radiotherapy. They express high levels of the ATP-binding cassette transporter proteins that efflux many chemotherapeutic drugs and they are inherently resistant to reactive oxygen species, thus rendering radiotherapy redundant. These properties are required by an isolated tumour cell to survive in an otherwise unfavourable environment.

**Senescence and related changes**

Cells that exit the cell cycle after stressful stimuli in a seemingly irreversible capacity become senescent. Many cellular stresses can induce senescence, which is identifiable by a multitude of morphological cellular features, most commonly the expansion of lysosomal beta-galactosidase levels. Senescence induction and maintenance are primarily mediated by the Arf/p53/p21 and p16/pRb tumour suppressor pathways, and also in response to progressive telomere shortening. Senescence may be partially rescued by tumour suppressor genes. Therefore, it is plausible that dormant cells may exist in a senescent-like state, still able to re-enter the cell cycle upon appropriate stimulation.

Quiescence is the reversible exiting of a cell from the cell cycle into a G0 arrested state. Factors that dictate cell cycle arrest or quiescence include the cyclins, p27 and p21, and this is the favoured explanation for the longevity in vivo of DTCs.

Hibernation has been explored as a mechanism to explain dormancy in the haematopoietic stem cell model. Haematopoietic stem cells in their bone marrow niche may mimic DTC in secondary sites. Cells in hibernation have the salient feature of altered lipid raft morphology through the PI3K-Akt-FoxO pathway, where the end products can block cell cycle progression. These lipid rafts act as an intermediary platform where cytokine signalling, membrane trafficking and cytoskeleton organisation are controlled. Several molecular mechanisms have been implicated in the haematopoietic stem cell hibernation theory e.g. Ang-1–Tie-2, Notch ligand–Notch signal.

Some of these pathways even cross over to other known cellular mechanisms, for example, the cell cycle regulator molecule p21, also active in quiescence and senescence.

Macroautophagy has been shown to be a mechanism for dormancy in primary tumours. In macroautophagy, cells undergo a bulk degradation of their intracellular organelles until favourable conditions return to allow proliferation to resume. The same phenomena may be replicated in DTCs as a survival mechanism. Cells that undergo macroautophagy in vitro switch to an apoptotic pathway. However in vivo, autophagic cells can persist in a dormant but viable state for prolonged periods.

**Other mechanisms**

Changes in genetic regulators may also play a part in the dormancy mystery. Methylation and histone deacetylation are important in regulating tumour promoters and suppressors. Potentially we can utilise knowledge from these known mechanisms to explain cellular interactions in clinically indolent disease. MicroRNAs are small non-coding RNAs that can regulate genes at both transcriptional and post-transcriptional levels. There is a growing number of dormancy associated microRNAs that have been shown to play a governing role in primary tumour dormancy, and their roles can be extrapolated to metastatic mechanisms as well. A single microRNA is able to alter the expression of multiple genes, and can play a key role in explaining how DTCs are regulated. Several microRNAs, including microRNA-16 and -19, are consistently found to regulate dormancy.

Interactions with the extracellular matrix are integral for the survival, growth, proliferation and invasion of tumour cells. Extracellular matrix of distant potential sites of metastasis may also affect DTC biology. Evidence of this in breast cancer is seen with BMP7, which is present in bone marrow and has been shown to induce tumour cell dormancy. Also, increased TGF-β in the microenvironment along with the stimulatory effects of mitogenic cytokines, may regulate the balance between dormancy and proliferation. Adhesion of tumour cells to the extracellular matrix is also required to enable growth, and inhibition of adhesion renders highly metastatic cells dormant.

A key concept in dormancy is the ‘switch’ from solitary tumour cells that may have survived for long periods, to an activated state resulting in the onset of clinically apparent metastasis. There are many proposed mechanisms for this switch, including activation of the endoplasmic reticulum stress signalling pathways that induce dormancy via p38/MAPK signalling. Alterations in the ERK/p38 activity ratio define primary tumour behaviour. A low ERK/p38 ratio correlates with slow tumour growth and a high activity ratio induces tumour proliferation.

This concept may be applicable in the metastasis context as well. Another proposed mechanism involves TGF-β2 signalling through TGF-β-R-II, resulting in p27-induced dormancy signalling.
Dormant cells as a target

Historically, the process of metastasis has been hypothesised as a linear growth pattern that should follow the behaviour of the primary tumour, and even somehow resemble the primary tumour. Both mathematical modelling and scientific studies have refuted this concept, and it now seems that there is a spectrum of behaviours of metastatic cells, possibly related to the timing at which these cells escape from the primary site. Cells undergo many changes to enable lodgement at a distant site, undetected by the surveillance systems of the host. Most studies of dormancy explore primary tumour dormancy as opposed to spontaneous metastatic dormancy, although the concepts can relate to both scenarios.

Dormant metastases are very difficult to study, and indirect methods are needed to further our understanding. The dormancy phenotype may be a culmination of multiple pathways and multiple phenotypes. Targeting dormant cells at the molecular level will be the next step in minimising recurrence rates and avoiding the corresponding clinical consequences.

Therapy development will depend on knowledge of the mechanisms that regulate dormancy. Using the proposed mechanisms discussed, there are two options for a therapeutic strategy against these evasive cells. Firstly, we can aim to impose dormancy on already disseminated tumour cells, forcing these cells to remain quiescent for the life of the host. Dormancy imposing therapies would need to be non-cytotoxic and applied chronically, aiming to prevent the switch from dormant disease to overt secondary growth. Alternatively, we could consider the opposite by preventing these cells from entering dormancy, so they become more sensitive to current therapies. A dormancy breaking treatment would then be used as an adjunct to current therapies. Both of these strategies require further understanding of the processes that control the dormancy of metastatic cells, an area of research being actively pursued by several groups, but still in its infancy.

Late recurrence is thought to be due to the survival of clinically undetectable metastases in patients. These are exceedingly difficult to study due to limitations in detection and understanding of their biology. Current hypotheses, as outlined above, need to be tested in relevant preclinical models. With increasing frequency of late recurrence, we must develop novel strategies to combat these dormant repositories of cells, both at the tumour mass level and at the tumour cell level. It is likely that we need to use a combination of mechanisms to target dormancy effectively.

References