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Research update

The role of phenotypic plasticity in the escape of cancer cells from targeted therapy

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ABSTRACT

Targeted therapy has proven to be beneficial at producing significant responses in patients with a wide variety of cancers. Despite initially impressive responses, most individuals ultimately fail these therapies and show signs of drug resistance. Very few patients are ever cured. Emerging evidence suggests that treatment of cancer cells with kinase inhibitors leads a minor population of cells to undergo a phenotypic switch to a more embryonic-like state. The adoption of this state, which is analogous to an epithelial-to-mesenchymal transition, is associated with drug resistance and increased tumor aggressiveness. In this commentary we will provide a comprehensive analysis of the mechanisms that underlie the embryonic reversion that occurs on targeted cancer therapy and will review potential novel therapeutic strategies designed to eradicate the escaping cells.

Contents

1. Introduction

For decades, the chemotherapeutic treatment of cancer has relied upon non-specific cytotoxic drugs which target rapidly dividing cells through the initiation of DNA damage, the stabilization of microtubules or the inhibition of nucleotide metabolism [1]. While being effective at killing cells with high proliferative rates, these therapies also target normal cell populations with rapid turnover, such as the cells of the hair follicles, the bone marrow and linings of the gut, leading to significant toxicity. A more effective approach to cancer therapy is the direct targeting of the genetic aberrations in the oncogenes that drive cancer development and progression - a strategy termed targeted therapy. As tumors typically have complex mutational profiles and a high level of redundancy in the pathways that drive growth and survival, targeted therapies work best in cases where the cancer is addicted to the activity of one oncogene for their growth and survival.

One of the first targeted therapies to be developed was imatinib, a Bcr-Abl kinase inhibitor/c-KIT inhibitor, with activity in chronic myeloid leukemia (CML) harboring the Bcr-Abl fusion protein

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and c-KIT mutant gastrointestinal stromal tumors (GIST), respectively [2,3]. Following this, targeted therapies have since been developed for many other cancers where driver oncogenes have been identified including EGFR mutant/expressing lung cancer/breast cancer/colorectal carcinoma, *BRAF*-mutant melanoma/hairy cell leukemia and ALK-mutant non-small cell lung cancer (NSCLC) [4–6]. Although drugs developed against these targets have revolutionized the treatment of many cancers, therapy failure is common with resistance usually associated with reactivation of the initial oncogenic pathway. The reactivation of signaling can be mediated through secondary mutations in the target gene of interest e.g. T790M mutations in EGFR, T315 mutations in Abl and L1196M mutations in ALK [7–9]. Escape from therapy can also be driven through the activation of bypass pathways, e.g. *NRAS*, *MEK* and *BRAF*-splice mutations in the case of BRAF inhibitor failure in melanoma and increased c-MET signaling in the case of erlotinib resistant lung cancer [10–12]. Although resistance is often genetically mediated, it does not always arise from pre-existing mutation bearing clones and often takes time to develop. The exact sequence of events that occur in between initial drug response and the emergence of fully resistant cells is not well understood. There is a growing body of evidence that suggests acquired resistance is non-genetically mediated, and is independent of secondary genetic events. This process of phenotypic adaptation, which allows tumor cells to dedifferentiate and adopt a more primitive embryonic state, is likely to contribute to therapeutic escape and may also increase the dissemination of the resistant tumor cells to other sites. There also seems to be some overlap between the therapeutically adapted state and that of so-called cancer "stem cells" – which also show phenotypic plasticity, slow growth and drug resistance. Defining and therapeutically targeting this phenotypic state is likely to be critical to the effective long-term management of many advanced cancers in which targeted therapy is used. In this review we will outline some of the mechanisms that underlie the phenotypic switch in cancer cells and will describe how this contributes both to therapy escape and drug resistance. The majority of our focus will be upon *BRAF* mutant melanoma and EGFR driven lung cancer.

2. The phenomenon of phenotype switching in normal physiology

Recent work has suggested that the phenotypic adaptation of cancer cells in the escape from therapy is akin to that of an epithelial-to-mesenchymal (EMT) transition, the widely conserved developmental process that permits the conversion of epithelial cells to a more mesenchymal-like state [13]. Under normal physiology, epithelial cells exist as organized sheets of cells that serve both as a protective barrier as well as well performing important secretory functions. Polarity and organization within the epithelial cell layers is achieved through E-cadherin based adherens junctions, tight junctions and connexins. The adhesion mediated through E-cadherin and tight junctions maintain the architecture and integrity of the epithelium and allow for the directed flow and transport of ions, nutrients and growth factors [14]. Mesenchymal cells, in contrast, are more supportive and play a role in extracellular matrix (ECM)-deposition, serve as tissue scaffolds and have critical roles in the repair of tissue damage and wound healing [14]. During embryonic development, some polarized epithelial cells dedifferentiate, downregulate their tight E-cadherin mediated adhesion and alter their morphology. These changes allow the cells to exit the epithelial environment and migrate to new locations, after which they redifferentiate, reacquire their ordered epithelial morphology and restore their cell–cell adhesions with other neighboring cells, a process known as mesenchymal to epithelial transition (MET).

The induction of the EMT is dependent upon complex functional networks involving at least 3 families of transcription factors which alter the expression of many genes important for decreased cell adhesion, increased cell migration and mesenchymal differentiation. One family, the Snail Zinc-finger transcription factors, including Snail1 and Slug, play a key role in EMT induction through their repressive effects upon E-cadherin through direct binding to the E-boxes of the CDH1 promoter [15]. Snail1 has also been shown to upregulate the mesenchymal markers fibronectin and vimentin [16] while both Snail1 and Slug have been implicated in metastasis [17]. Another group of EMT-associated transcription factors are the basic helix–loop–helix (bHLH) family members Twist1 and Twist2. Both Twist1 and Twist2 have been implicated in EMT through their ability to induce a stem-like state and to repress E-cadherin expression through the induction of Snail family transcription factors [18]. The final group of EMT-associated transcription factors is the homeobox family, comprising ZEB1, ZEB2 and NANOG. Both ZEB1 and ZEB2 decrease the transcription of E-cadherin [19], with ZEB1 being further shown to repress miRNA-203 and miRNA-200 expression [20]. ZEB2 also has a role in the downregulation of transcripts encoding P-cadherin, claudin 4, and the tight junction protein 3 (*ZO-3*) [21]. NANOG regulates EMT through decreasing levels of E-cadherin expression while increasing the expression of vimentin, β-catenin and Snail [22,23].

The role of EMT in cancer is well known and represents one mechanism by which non-motile, polarized, epithelial cells dedifferentiate and acquire a more invasive and mobile phenotype [24]. Induction of these EMT-associated transcription factors can also arise following microenvironmental signals including transforming growth factor-β (TGF-β) superfamily, the WNT family, and growth factors from the fibroblast growth factor (FGF) family. Among these, the TGF-β pathway is a primary driver of EMT [25] with the ability to induce all three EMT transcription factor families including Snail1/Slug, ZEB1/2, and Twist1 [26]. TGF-β family members often remain in a latent form through their binding to several binding factors and their activation occurs by binding to membrane receptor serine/threonine kinases I/II, serving as a connector to Smad proteins [27]. TGF-β/Smad signaling induces EMT in cooperation with other signaling pathways, which can include Ras activation [28] and Wnt/β-catenin [29]. TGF-β itself is secreted by nearby stromal fibroblasts in response to tumor-derived signals or other stresses [30]. The fibroblasts are important regulators in this context and they can modulate the expression of smooth muscle actin (α-SMA), FGF and collagen which also lead to EMT induction [31]. While TNFα plays a vital role in the regulation and homeostasis of immune cells, it has also been shown to induce expression of EMT transcription factors such as Snail1 via the activation of NF-κB [32] and Twist1 via activation of IKK-β [33]. TNFα also has been shown to decrease the expression of E-cadherin and upregulate vimentin [34]. Within a tumor, EMT does not occur uniformly, with a greater proportion of mesenchymal cells being observed at the leading edge and in areas of hypoxia. The role of EMT in tumor progression has been widely discussed elsewhere, and the reader is directed to the following excellent articles for a more thorough discussion of this topic [35–38].

3. Phenotypic adaptation as a mediator of drug resistance

One of the major unanswered questions in the study of drug resistance is whether therapeutic escape results from the selection of pre-exiting rare clones of cells harboring resistance mutations or instead emerges from an evolutionary process in the cells that escape the initial therapeutic insult, allowing the later emergence of resistance conferring mutations. As the majority of patients receiving targeted therapy show at least some level of response,