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Review

The architect who never sleeps: Tumor-induced plasticity

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ABSTRACT

Tumor cell plasticity is an event that has been observed in several malignancies. In fact, most of the solid tumors are characterized by cellular heterogeneity and undergo constant changes as the tumor develops. The increased plasticity displayed by these cells allows them to acquire additional properties, enabling epithelial-mesenchymal transitions, dedifferentiation and the acquisition of stem cell-like properties. Here we discuss the particular importance of an inflammatory microenvironment for the bidirectional control of cellular plasticity and the potential for therapeutic intervention.

1. Introduction

A tumor originates from the clonal expansion of a single cell that has acquired the ability of extensive self-renewal and unlimited proliferation. Surprisingly however, a tumor is never composed of completely identical cells; in fact most of the solid malignancies are characterized by heterogeneity and are undergoing constant changes as the tumor develops. Importantly, it is not only the tumor cell that is highly plastic, but also the tumor microenvironment. The bidirectional regulation that exists between tumor cells and their environment is the most important determinant of tumor plasticity. Since tumors undergo continuous alterations as they progress, the tumor site can be considered as a place where a never-ending deconstruction and reconstruction takes place and the tumor itself is the architect.

This extreme plasticity which characterizes malignant growth can be induced both by tumor cell-intrinsic- and extrinsic factors. Among the cell-intrinsic factors that can induce a highly plastic phenotype are mutations in oncogenes and tumor suppressors. These mutations can alter not only the morphology, metabolic state, proliferative capacity or the motility of the cell, but also determine its interaction with the other cells in its vicinity. Therefore, the tumor cell has the ability to alter its microenvironment by direct cell–cell contact or by release of paracrine factors. This way the tumor cell is able to alter the tumor composition by inducing the formation of new blood vessels, recruiting new cell types and restructuring the extracellular matrix. On the other hand, the tumor microenvironment has a very profound effect on the tumor cell plasticity as well. Depending on the environment in which the tumor cell resides it can adopt different phenotypes and behaviors contributing to intratumoral heterogeneity. However, if the cell leaves its established microenvironment or the environment changes, the tumor cell can adopt completely new properties.

Two important mechanisms controlling tumor cell plasticity significantly contribute both to tumor initiation and progression. During tumor initiation, dedifferentiation and acquisition of stem cell-like characteristics increases the number of tumor-initiating cells and favors self-renewal. At later stages, these stem cell-like cells are suggested to play an important role in therapy-resistance and metastases. Similarly, during tumor progression, the induction of epithelial-mesenchymal plasticity (EMP), including both epithelial to mesenchymal – and mesenchymal to epithelial transition, enables tumor cells with the ability to migrate to distant organs and establish metastases. Both EMP and dedifferentiation can therefore be considered as distinct types of tumor-plasticity that result in a population of tumor cells that contribute to a more aggressive phenotype. Although EMP and dedifferentiation are independently controlled events they also share certain common triggers and regulatory mechanisms.

2. On the move

One important phenomenon in tumor cell plasticity is the ability of the epithelial tumor cells to acquire a more mesenchymal phenotype and when needed return to the original epithelial state. By hijacking the mechanisms of embryonic developmental programs, epithelial to mesenchymal transition (EMT) and

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mesenchymal to epithelial transition (MET), tumor cells gain capabilities to invade surrounding tissues, migrate to- and colonize distant organs. The ability of the tumor cells to readily transit between epithelial- and mesenchymal states can be referred to as epithelial-mesenchymal plasticity [1].

Epithelial and mesenchymal cells are recognized by their unique morphology and tissue organization. They differ in several important aspects such as polarity and cell–cell contacts. In contrast to epithelial cells, which usually form continuous and cohesive sheets that line the cavities throughout the body, mesenchymal cells embed themselves inside the extracellular matrix (ECM) and rarely establish tight junctions with surrounding cells. During embryonic morphogenesis cells need to migrate to adjacent tissues and even travel long distances inside the embryo. This process is only possible due to the activation of the epithelial to mesenchymal transition program, which allows stationary epithelial cells to become dynamic and to move during the developmental morphogenesis [2,3]. In summary, loss of cell junctions and apico-basal polarity together with the acquisition of mobility are the steps which epithelial cells are submitted during EMT. Importantly, when those cells reach their final destination the EMT program must be reversed, with cells returning to their original epithelial phenotype, undergoing mesenchymal to epithelial transition, or MET. Only then, they can start to proliferate and give rise to different tissues and organs [1,4].

Intriguingly, the EMT and MET process are also found in non-homeostatic situations. Research on this topic suggests that EMT and MET are restarted in many diseases, with a fully activated pattern in malignant processes and its dissemination to distant organ – metastasis [5–7].

EMT is often adopted by cancer cells, providing them with a highly invasive and migratory phenotype. The contribution of EMT in promoting cancer cell invasion and metastasis has been documented in many carcinoma types, including those arising in colon, ovary, breast, lung, prostate and head and neck [4,8,9]. It is during EMT – and later during MET – that cancer cells invade the surrounding tissue, enter the microvasculature (intravasation), translocate through the bloodstream, exit from the blood vessels (extravasation), survive in a different microenvironment and finally colonize it, forming a secondary tumor site, the metastasis [10,11].

To initiate this process the epithelial tumor cells must pass through important molecular changes. Genes encoding cell-junction proteins, such as E-cadherin, alpha-catenin and gamma-catenin are downregulated. Among them, E-cadherin is regarded as the main marker of the epithelial state [2,3]. E-cadherin transcriptional repression, methylation, protein phosphorylation and degradation have been already observed in response to EMT-inducing signals by several groups [12–17]. While E-cadherin expression is lost, the level of the mesenchymal-specific marker, vimentin, increases, due to the switch of cytokeratin to vimentin filaments (Table 1 lists some of the most common markers used to define EMT in tumors).

It is generally accepted that EMT is a prerequisite for invasion and metastatic dissemination, however distant metastases generally present an epithelial morphology [18,19]. But why would tumor cells revert to an epithelial phenotype to fully establish a macrometastasis? In vitro studies showed that on reason might be the EMT-associated growth arrest, and since colonization of the metastatic site demands a robust tumor cell proliferation, reversion of EMT might provide growth advantages [20,21]. Although not universally accepted in cancer research, the presence of a mesenchymal to epithelial transition has been elegantly supported in vivo by recent studies. Using a spontaneous squamous cell carcinoma model in mice overexpressing skin-specific Twist-1, Tsai et al. raised the possibility that tumor dormancy could be due to the inability of disseminated tumor cells to revert the EMT status and proliferate [22]. Moreover, Gao and colleagues, demonstrated that versican knockdown in bone marrow cells significantly impaired lung metastases in vivo, without impacting their recruitment to the lungs or altering the immune microenvironment. Versican, an extracellular matrix proteoglycan, stimulated mesenchymal-epithelial transition of metastatic tumor cells by attenuating phospho-Smad2 levels, which resulted in elevated cell proliferation and accelerated metastases [23]. Consistently with both works, Ocaña et al. showed that downregulation of an EMT marker, Prrxl in human breast cancer cells was necessary for effective lung metastasis colonization [24].

It is necessary to point out that induction of EMP in cancer cells is not necessarily an intrinsic phenomenon during tumor progression, yet, it has been suggested as a response to extracellular signals from the tumor microenvironment [25–32]. Several ligands activate and maintain cancer cell plasticity, either in autocrine or paracrine manners. TGF-β signaling appears to be one of the major inducers of plasticity and EMT during embryonic development and cancer progression [33,34] TGF-β signaling has been demonstrated to result directly in the epigenetic regulation of downstream target genes. SMAD2 and SMAD3 associate with certain epigenetic regulators, such as TRIM33, which displace repressive histone modifications, creating a poised chromatin structure that can be accessed by transcriptional regulators [35] The EMT transcription factor Snail represses the expression of E-cadherin and thereby confers a fibroblast-like behavior onto epithelial cells that includes increased motility. This process occurs at the invasive

Table 1
Commonly used EMT markers in cancer. TGF-β: transforming growth factor β; αSMA: alfa smooth muscle actin; MMP: matrix metalloproteinase; NF-κB: nuclear factor kappa B; Sox10: SRY (sex determining region Y) box 10; SMAD: contraction of Sma and Mad (Mothers against decapentaplegic); PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B.

EMT markers in cancer
Decreased expression
• E-cadherin
• Mucin-1
• Cytokeratins (e.g. CK19, CK18, CK8)
• Occludin
• Desmoplakin
Increased expression
• Vimentin
• TGF-ß
• N-Cadherin
• αSMA
• Fibronectin
• Vitronectin
• Collagen I and III
• MMP2, MMP3 and MMP9
• Thrombospondin
• Tenascin C
Deregulated or altered cell localization
• ß-catenin
• NF-κB
• Snail
• Slug
• Twist
• Sox 10
• SMAD 2 and SMAD 3
• PI3K/AKT

front of tumors, the same site where tumor infiltration by tumor-associated macrophages (TAMs) takes place. An elegant study by Wu et al. links these events by demonstrating that TAMs-derived TNF-α via NF-κB leads to the stabilization of Snail, which is otherwise a highly unstable protein. Knockdown of Snail expression inhibits inflammation-induced breast cancer cell migration and invasion in vitro and metastasis in vivo, suggesting that EMT is a dynamic process controlled by an inflammatory microenvironment [36,37].

The importance of a NF-κB-dependent inflammatory microenvironment for induction of EMT, enabling invasion and lymph node metastasis was recently demonstrated in a model of carcinogen-induced colorectal tumorigenesis [38]. Loss of p53 in the intestinal epithelial cells leads to a change in the composition of tight junctions and expression of mucins, which impairs the intestinal epithelial barrier resulting in a chain of events that promote tumor progression. As a consequence of the increased intestinal permeability and enhanced delivery of bacterial products, intestinal epithelial cells (IEC) activate inflammatory NF-κB signaling and start to produce diverse chemokines. These chemokines recruit myeloid cells to the tumor site, where they produce several NF-κB-dependent pro-tumorigenic cytokines. Moreover, NF-κB activation in IEC controls expression of Twist, which is essential for the induction of EMT (Fig. 1). Interestingly, deregulation of miR-34 that is also controlled by p53 may further contribute to the invasive phenotype. MiR-34 suppression can be mediated by the inflammatory tumor microenvironment via an IL-6/STAT3 loop [6].

Intriguingly, in some cases epithelial-mesenchymal plasticity is closely associated with the acquisition of stem cell-like characteristics. Human basal breast cancer cells are highly plastic and can revert from a non-cancer stem cell (CSC) state to a cancer stem cell state by upregulating the EMT transcription factor Zeb1. Interestingly, the promoter region of Zeb1 is in a bivalent state in the non-CSC population and therefore it can readily switch to an active configuration in response to stromal TGFβ [39]. Under normal circumstances the expression of Zeb1 is controlled by the miRNA-200 family. Downregulation of miRNA-200 members, which is often the case in many cancers, induces EMT and very importantly a cancer stem cell phenotype [40]. Similar bidirectional negative feed-back loop exists between Snail and mir-34 [41] and mir-34 has been also shown to suppress sternness [42,43]. On the contrary, evidence can be also found that it is the epithelial phenotype that is required for the cell to acquire stem cell functions. This is supported by the fact that generation of induced pluripotent stem cells (iPCSs) from mouse embryonic fibroblast requires the transition to the epithelial state at the initial phase of the reprogramming process and is orchestrated by BMP signaling [44]. Similarly, downregulation of potent EMT inducers is associated not only with the acquisition of an epithelial- but also- of a stem cell phenotype [24,45]. Irrespectively of the question if it is the epithelial or mesenchymal state that is associated with the stem cell phenotype it is very likely that cancer cells can readily switch between these states in response to external stimuli, which might help them to effectively adopt to various environments.

3. Catching stemness

Most cancers arise by the step-wise accumulation of genetic and epigenetic hits in specific genes that endow the mutated cell with growth advantage. When aberrant genetic changes are introduced into a stem cell it is more likely that these genetic and epigenetic alterations will lead to tumor development. Stem cells are long-lived and self-renewing cells and thus have the ability to accumulate and propagate such mutations and therefore are good candidates for the cell of origin in many tumors. Studies using genetically engineered mice provide firm evidence for the existence and importance of cancer stem cells in tumor initiation and growth. Alteration in the Wnt signaling by mutations in *APC* or *CTNNB* represents one of the earliest steps of colorectal carcinogenesis. Introduction of a stabilized β-catenin allele or deletion of *Apc* in intestinal stem cells (ISCs), leads to rapid tumor development and provides evidence for the role of ISCs in tumor initiation [46–50]. Moreover, Schepers and colleagues have shown that stem cells contribute not only to the initial stages of tumor development, but actively participate in the maintenance of the tumor [50].

The tumor microenvironment can significantly contribute to stemness by activating or expanding the stem cell pool. Inflammation is known to increase the number-of wnt-active and tumor initiating cells [51], therefore inflammatory conditions in the tumor microenvironment might enhance tumor initiation by an effect on the stem cell compartment. Inflammation induces the

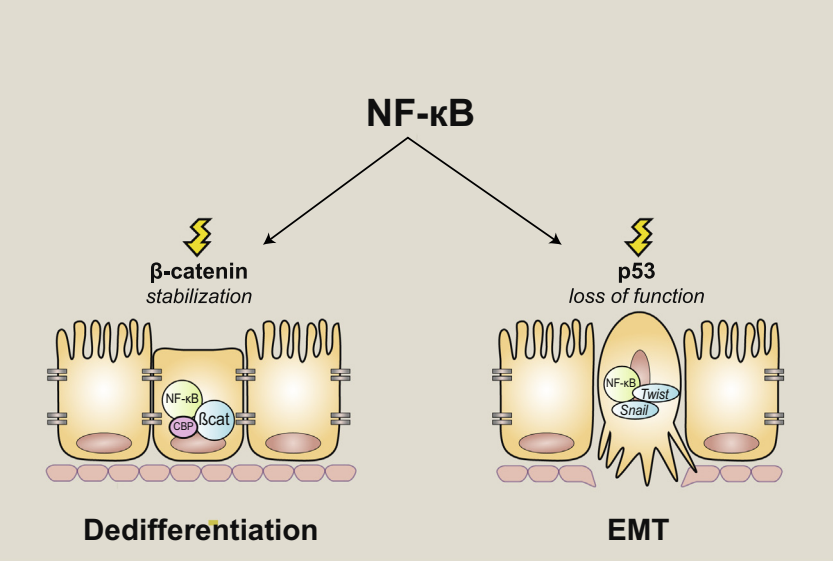

Fig. 1. NF-κB signaling exerted effects. High wnt-activity and concomitant NF-κB activation induces dedifferentiation and acquisition of stem cell-like properties. Simultaneously, NF-κB-dependent inflammatory microenvironment induces EMT promoting invasion and lymph node metastasis.

activation of the Akt/PI3K pathway which leads to the subsequent Akt mediated phosphorylation and nuclear translocation of β-catenin [52]. Phosphorylation of β-catenin by Akt probably governs the activation of the of the stem cell compartment [53]. Moreover, Lgr5 expression levels are increased in patients with ulcerative colitis and Crohn's disease [52].

However, stemness might be not a fixed state of the cell and a scenario in which cells can enter and leave the CSC-like state (depending on their niche and environmental signals) is conceivable. If the acquisition of the stem cell fate was a dynamic and reversible process it is unlikely to be mediated solely by irreversible genetic changes such as mutations. Most probably flexible mechanisms such as epigenetic regulation of the gene expression are dominant determinants of the cell stemness (Fig. 2). Therefore, we suggest that, if exposed to certain environmental stimuli, even differentiated cells can interconvert into a stem cell-like state during a neoplastic process. Differentiated cells can regain self-renewing and multipotent properties and thus can behave as cancer stem cells. Recently, we have shown that differentiated enterocytes can regain stem cell like characteristics and initiate intestinal tumorigenesis in vivo. Interestingly, this phenomenon was dependent on high wnt activity and concomitant NF-κB activation, suggesting that inflammatory conditions in the tumor may not only expand the stem cell pool, but also induce dedifferentiation and acquisition of stem cell like properties (Fig. 1) [54]. Importantly, in mixed lineage leukemia it is NF-κB activity, which is required to maintain the aberrant histone modifications and the induced stem cell program, suggesting that inflammatory conditions regulate stem cell fate via epigenetic reprogramming, most likely in cooperation with genetic changes in important tumor suppressor and oncogenes (Fig. 2) [55].

Another argument in the support of the reversibility of the differentiated state is the fact that the generation of induced pluripotent stem cells does not require genetic changes and it can be induced by the ectopic expression of a small number of transcription factors [56]. The embryonic reprogramming factor Oct4 is expressed in many cancers and expression of Oct4 promotes melanoma cell dedifferentiation and acquisition of a stem cell like fate by inducing the expression of other embryonic transcription factors such as Nanog and Klf4. Interestingly, Oct4 expression is again regulated by the microenvironment as hypoxia strongly elevates Oct4 levels providing a possible explanation how hypoxia can induce a more aggressive phenotype [57].

Considering the major effect of the tumor microenvironment on dedifferentiation, the particular niche in which the tumor cell resides in might be one of the most important determinants of its stemness. In colorectal tumors stromal cells and vessels are not equally distributed and tumor cells located at the stroma-rich regions of the tumors might therefore receive more signals that direct them toward a stem cell-like state than tumor cells that are located at stroma-poor regions. Cancer-associated fibroblasts (CAFs) which are enriched at the invasion fronts of colorectal tumors produce HGF, OPN and SDF-1 to activate the c-Met/Akt and wnt pathway crosstalk in the surrounding cells. Strong activation of the wnt pathway, which is also very important for the normal intestinal stem cells, results in the conversion of the differentiated cells into a more stem cell-like state [58,59]. Similarly, in glioblastoma, nitric oxide that is produced by the tumor vasculature activates Notch signaling and induces the expression of the stem cell marker nestin in the cells located in the perivascular niche [60]. Induction of dedifferentiation by inflammation or other environmental stimuli has also major

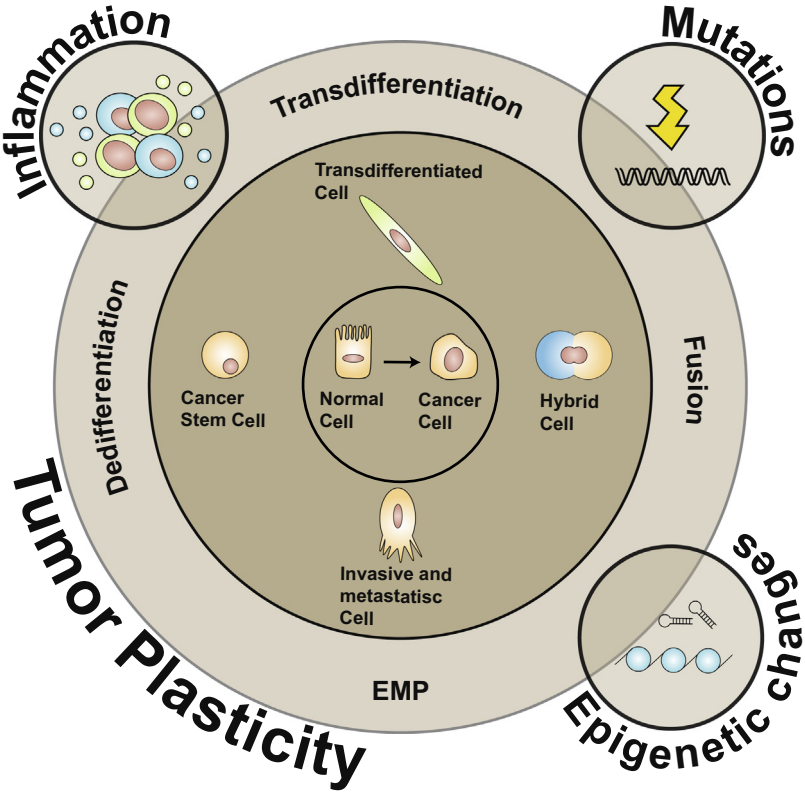

Fig. 2. Tumor plasticity. EMP, dedifferentiation, transdifferentiation and cell fusion can be viewed as the four major sources of tumor plasticity and heterogeneity during tumorigenesis. EMP enhances invasion, and metastatic dissemination. Dedifferentiation of mature cells increases stemness and is believed to contribute to therapy resistance. Transdifferentiation causes one cell type to interconvert into a different cell type and cell fusion generates hybrid cells with both epithelial and immune cell-like properties. All process share some common characteristics and similar regulatory mechanisms. The three small circles represent the three major inducers of tumor-plasticity. Mutations in most important tumor suppressors and oncogenes, epigenetic modifications and the inflammatory microenvironment are the most potent inducers of tumor cell plasticity.

therapeutical consequences as it can induce reversible downregulation of antigens that are recognized by the immune system. These tumors can evade from anti-tumor immune responses and resist adoptive T cell therapies [61].

Dedifferentiation of fully differentiated cells is not uniquely restricted to malignant cells, but upon damage or imbalance in tissue homeostasis, also normal mature cells can reprogram into a stem cell. In the airways epithelial stem cells reside at the basal layer of the epithelium and produce multiple lineages of differentiated cells. Ablation of these airway basal stem cells induces the dedifferentiation of luminal secretory cells into stem cells that are morphologically and functionally indistinguishable from the original basal stem cells [62]. Similarly, radiation induced loss of $Lgr5^+$ cells in the intestinal crypts directs Dll^+ secretory precursors to regain stem cell properties and replenish $Lgr5^+$ stem cells [63]. These findings suggest that a certain level of plasticity is required to maintain normal tissue homeostasis and to activate and accomplish a proper regenerative program after injury. Most probably to drive tumor initiation and progression cancer stem cells hijack those mechanisms of the normal stem cells which they use to restore tissue homeostasis. However, while dedifferentiation of the normal tissue stem cells is a transient and most likely strictly regulated process, in cancer stem cells these regulatory pathways and checkpoints are missing or can be overcome. Therefore, by studying regenerative responses in normal tissues we can possible gain a better insight into tumor plasticity induced by dedifferentiation.

Apart from dedifferentiation also transdifferentiation can be observed during tumorigenesis (Fig. 2). Tissue metaplasia, a switch from one tissue type to another tissues type, requires the transdifferentiation of tissue cells. Intestinal metaplasia of the gastric epithelium is one of the earliest steps in gastric cancer and metaplastic lesions of the stomach develop into invasive cancer via the metaplasia-dysplasia-carcinoma sequence. The most frequent cause of intestinal metaplasia is Helicobacter pylori-induced gastritis. It activates wnt/β-catenin signaling and induces the expression of the homeobox transcription factor Cdx1, a master regulator of the gut development and homeostasis, which under physiological conditions is never expressed in the stomach [64]. Cdx1 expression in the stomach activates Klf5 and Sall4 reprogramming factors and leads to an increase in the intestinal stem cell markers such as Lgr5 and Bmi1, suggesting that transdifferentiation of gastric epithelial cells into intestinal-like cells occurs via a more dedifferentiated state [65]. Besides the important finding that transdifferentiation requires reprogramming these data also pinpoint the fact that the whole process is induced by environmental stimuli. Microenvironmental factors, especially inflammation, have been also shown to contribute to Barret esophagus (BE) and esophageal adenocarcinoma (EAC). In BE the stratified epithelium of the esophagus is replaced by metaplastic intestinal-like epithelium, that may originate from $Lgr5^+$ cardia cells which in response to NF-κB-dependent cytokines, such as IL-6 and IL-1β, migrate up to the esophagus and serve as cell of origin for BE and EAC [5]. Metaplasia or transdifferentiation also occurs in early stages of pancreatic ductal adenocarcinoma (PDAC) development. Although the cell of origin of pancreatic cancer has not been identified yet, the requirement of acinar-to-ductal metaplasia (ADM) for PDAC is well defined. Activation of oncogenic K-Ras in combination with Notch in mature acinar cells induces pronounced ADM which is followed by the appearance of pancreatic intraepithelial lesions [66]. Similar to intestinal metaplasia inflammation has a very profound effect on ADM and pancreatic cancer and it is essential for the induction of PDAC in adult mice [67]. One possibility how inflammation contributes to ADM and subsequent PDAC is the release in inflammatory cytokines by the infiltrating macrophages and activation of NF-κB in the acinar cells [68]. In addition to that, within the process of malignant transformation pancreatic cells not only elicit the production of growth factors, cytokines and chemokines by the surrounding environment but they can, as a consequence of transdifferentiation, comprise the main source of those molecules [69].

Last but not least, fusion of cells of hematopoietic origin with tumor cells could also significantly contribute to tumor heterogeneity (Fig. 2). Fusion of macrophages with tumor cells of Apc^{Min} mice results in the formation of a new population of tumor cells that share both macrophage- and tumor cell characteristics [70]. The fusion of the epithelial tumor cells with immune cells is believed to confer a more stem cell-like and migratory phenotype to the fusion-hybrid cells and therefore enhance the metastatic spread of these cells [71,72].

4. Conclusions

In the last decade many new therapeutic strategies and sensitive diagnostic tools have been developed to cure cancer. Despite the efforts that have been made there is still an increasing number of patients that succumb to this disease. Resistance to therapies that occurs in most patients can be in part a consequence of tumor plasticity. By changing their phenotype or by altering the microenvironment tumor cells can evade therapies. Therefore, a therapeutic approach that blocks plasticity would be of great interest. Inflammation represents a central regulator of tumor-associated plasticity. Many examples have demonstrated that the NF-κB signaling network is central to cancer-associated inflammation. NF-κB signaling has roles both in the tumor-infiltrating cells and the cancer cells themselves [73]. In infiltrating cells NF-κB signaling promotes the production of inflammatory cytokines, many of which have the potential to induce tumor cell plasticity either by EMP or by reversing the cells to a less differentiated state [38,61,74,75]. Therefore, NF-κB or its downstream target cytokines and chemokines inducing and maintaining an pro-inflammatory microenvironment may represent very powerful targets to interfere with several aspects of tumor plasticity.

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