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Cell Cycle News & Views

Control of cancer-associated fibroblast function by oxidative stress: A new piece in the puzzle

Comment on: Fiaschi T, et al. Cell Cycle 2013; 12:1791–801; PMID:23656776; http://dx.doi.org/10.4161/cc.24902

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Fibroblasts are found in various proportions across the spectrum of carcinomas, constituting, in many cases, the preponderant cell population of the tumor stroma. Data from co-culture and reconstitution experiments indicate that fibroblasts have a prominent role in defining the rate and extent of cancer progression and metastasis. In a recent issue of *Cell Cycle*, Fiaschi et al. described a new mechanism by which cancer-associated fibroblasts (CAFs) promote metastatic dissemination.[1] They observed that CAFs have a major contribution to extracellular acidification through de novo carbonic anhydrase IX (CAIX) expression and activation upon stimulation by tumor epithelial cells. Importantly, such activation is observed in vitro as well as in CAFs isolated from patients bearing aggressive prostate carcinomas, confirming the physiopathological relevance of these observations. Thus, although the role of CAIX in epithelial tumor cells has been clearly established previously,[2] those observations indicate that activation of CAIX in CAFs drives the extracellular acidification of prostate carcinoma microenvironment, and that CAIX represents a new marker for CAFs.

Since the hypoxia-inducible factor 1 (HIF-1) has been reported as a master CAIX regulator in tumor epithelial cells, the authors next investigated the effect of HIF-1 inhibition by pharmacological inhibitors or RNAi silencing. CAIX upregulation in stromal fibroblasts required a ROS-dependent stabilization of HIF-1 in normoxia. These findings are in line with previous reports describing redox-based HIF-1 stabilization under normoxic conditions,[3] especially in CAFs associated with breast or prostate carcinomas.[4,5]

What are the functional consequences of CAIX-mediated extracellular acidification? CAIX activation resulted in increased MMP2 and MMP9 activity, leading to activation of EMT (as characterized by E-cadherin decrease, morphological features, and invasiveness) by tumor epithelial cells. Similarly, acidification of the extracellular medium greatly increased CAFs ability to drive EMT program in tumor cells. Importantly, CAIX-silenced CAFs were unable to support tumor outgrowth and lung metastasis formation upon co-injection with prostate tumor epithelial cells, confirming in vivo that CAIX is mandatory for the EMT process and metastatic dissemination.

This study is the first report of the role of CAFs in tumor microenvironment acidification, a salient feature commonly associated to tumor epithelial cells showing metabolic reprogramming toward the Warburg phenotype. Several biological processes are likely to contribute to such CAFs-mediated acidification. They include CAIX upregulation (described here) as well as increased glycolytic activity. Indeed, in contact with prostate tumor cells, CAFs undergo a mitochondrial oxidative stress and a metabolic reprogramming toward a Warburg phenotype, resulting in dramatic production of lactate that is extruded in the extracellular milieu together with H^+ ions. CAIX upregulation in stromal cells is under the control of the HIF transcription factor, which accumulates through a ROS-dependent mechanism. The factors modulating ROS production in CAFs have not been investigated in Fiashi's study and still remain unknown. However, one can argue that H_2O_2, a diffusible ROS involved in intercellular communication,[6] might be produced by tumor epithelial cells and involved in CAIX activation in CAFs. In that matter, it has been reported that non-phagocytic NADPH-oxidase (Nox) enzymes are overexpressed at the plasma membrane of tumor epithelial cells and might contribute to the production of H_2O_2. Alternatively, MMPs, such as MMP3, have been shown to modulate activity of the mitochondrial respiratory chain, subsequently increasing cellular ROS content.[7] It has also been shown that stimulation of receptor tyrosine kinase by growth factors, such as epidermal growth factor, is associated with ROS generation,[8] suggesting that such growth factors might also contribute to redox-based HIF-1 stabilization. Lastly, when tumors reach a certain size, inadequate oxygen delivery leads to hypoxia. In response, new vessels are formed, allowing tissue reoxygenation. However, tumor blood vessels are mostly disorganized and leaky, and oxygen within tumors varies both in space and time. Cycles of hypoxia and re-oxygenation can increase ROS production, which can stabilize HIF-1 and further amplifies acidic stress.

Several CAIX inhibitors are currently in clinical development in solid tumors, including breast and kidney cancer. In this line, the current study supports efforts to treat cancer patients with CAIX inhibitors: as CAFs are non-transformed genetically stable cells and therefore less likely to acquire drug resistance, they represent ideal pharmacological targets. Further studies will be required to investigate combination therapies with conventional chemotherapeutic treatments.

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miR-155 meets the JAK/STAT pathway

Comment on: Kopp KL, et al. Cell Cycle 2013; 12:1939–47;
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microRNA-155 (miR-155) is one of the most studied miRNAs and the first one to be described as oncogenic.[1] Encoded by its host gene, MIR155HG, also referred to as BIC (B cell integration cluster), was first found accumulated in B-cell lymphoma by Eis et al.[2] Soon after, miR-155 overexpression was observed in a long list of both hematological and solid-tissue cancers, where it was found to promote genomic instability, proliferation, and survival of malignant cells.[3] These properties have led to miR-155 now being referred to as an "oncomiR". Simultaneously, data emerged suggesting that miR-155 plays an absolutely crucial role in development and activation of several types of immune cells, including B and T cells.[4] Thus, this little molecule is one of those players that make apparent the link between inflammation and cancer, postulated by Virchow so many years ago: transient elevation of miR-155 levels is observed and necessary for functioning of immune cells; however, a chronically increased expression of miR-155 is often found in cancer, and miR-155 expression alone is sufficient to trigger malignant transformation.[1-3] The idea of miR-155 as a bridge between inflammation and cancer is supported by the fact that miR-155 can be induced by several inflammatory stimuli,[3] and among its targets are tumor suppressors and anti-inflammatory molecules.[5] However, miRNAs function as fine-tuners of protein expression and therefore the signals triggering expression of a miRNA are tightly regulated and seem to be highly context-dependent. Induction pathways as well as target genes vary among different cell types, tissues, or diseases. To understand the oncogenic properties of miR-155 expression, it is important to elucidate this variety of pathways in order to determine which stimuli regulate this miRNA that has the potential to transform cells and seems to be deregulated in so many cancers. Initially, a large fraction of the research on miRNAs primarily comprised expression profiles, revealing differential expression of specific miRNAs in various disease settings. Only recently research has started to focus on regulation and function of miRNAs. One would think that, miR-155 being a necessary component required for normal immune function, more studies would have investigated the specific influence of key cytokines on its expression, but surprisingly little is known about miR-155 modulation in response to cytokine stimulation. A new study by Kopp et al.[6] now connects expression of miR-155 to JAK/STAT signaling. In a recent issue of *Cell Cycle*, Kopp et al. report that the MIR155HG is a transcriptional target of STAT5, a well-known malefactor in the pathogenesis of lymphoma as well as several other cancers. Kopp et al. show that in cutaneous T-cell lymphoma (CTCL) cells, STAT5 (persistently activated in a majority of patients) directly binds to the BIC promoter and induces miR-155 expression. In CTCL T cell lines and primary cells, IL-2 or IL-15-induced (IL2Rb cytokines that signal through STAT5) constitutive STAT5 activation results in an increased transcription of BIC. Conversely, knockdown of STAT5 entails a decrease in miR-155 in the malignant cells as well as inhibits their proliferative capacity, an effect that is paralleled by direct inhibition of miR-155. This points toward a functional role of miR-155 in maintaining malignant proliferation. Furthermore, the fact that STAT5-dependent miR-155 induction was also observed in non-malignant T cells and PBMCS points toward a general (non-CTCL-specific) pathway of miR-155 regulation. Chronic inflammation involving a disturbed expression of miRNAs, including miR-155, is a hallmark of CTCL,[7,8] and the JAK/STAT pathways have long been known to be crucial players in the pathogenesis of leukemia and lymphoma. This recent study by Kopp et al. connects expression of an oncogenic miRNA to cytokine (or aberrant) signaling of the JAK/STAT pathway and sheds light on the particular role of STAT5 in the induction of oncogenic molecules, thereby further clarifying the link between inflammation and cancer. It remains to be investigated if the same mechanism is also working in other malignancies.

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Keeping each other in check: A reciprocal relationship between cytokines and miRNA

Comment on: Kopp KL, et al. Cell Cycle 2013; 12:1939–47;
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Cytokines are a large group of secreted messenger molecules that are produced by different cell types, including cells of the immune system and tumor cells. Cytokine signaling is necessary for successfully mounting as well as dissolving an immune response. Cytokines signal through their specific receptors. Signal transduction from these receptors happens through the JAK/STAT pathway that recently celebrated its 20th anniversary of discovery. The activation of a kinase (JAK1, JAK2, JAK3, or TYK2) downstream of the cytokine receptor results in phosphorylation and dimerization of the STAT transcription factors (STAT1–4, STAT5A, STAT5B, and STAT6) that translocate to the nucleus and regulate transcription of their target genes. Apart from transcriptional regulation, cytokine signaling is regulated through feedback loops by the STAT targets themselves, e.g., suppressor of cytokine signaling (SOCS) proteins or interleukin receptors (as reviewed in ref. 1). In chronic inflammation, autoimmune diseases as well as cancer aberrant cytokine signaling are often observed. In this regard, in chronic skin inflammation, T cells from psoriatic skin lesions display hypersensitivity for IFNα with enhanced STAT3 signaling due to a deficiency in SOCS3. Likewise, malignant T cells in skin lesions from mycosis fungoides (the most common form of cutaneous T-cell lymphoma [CTCL]) become resistant to IFNα due to an aberrant expression of SOCS3.[2-4]

In the last couple of years, however, yet another layer of cytokine regulation is being unraveled: miRNAs. miRNAs are small noncoding RNAs that can bind to the 3′UTR of mRNAs of cytokines or that of other signaling components, thereby inhibiting their translation. Two important examples are miR-155, a major oncogenic miRNA that targets SOCS1, as well as miR-203, a skin-specific miRNA that targets SOCS3 (as reviewed in ref. 5).

Although so far virtually overlooked, the regulatory relationship between cytokines and miRNA seems to be reciprocal: not only do miRNAs target cytokine mRNA, and thereby regulate cytokine expression, the cytokine signaling has likewise an impact on miRNA expression. Thus, it seems crucial to investigate how altered cytokine patterns, as for instance in inflammation and cancer, influence miRNA expression patterns. In this regard, Kopp et al., in an exciting study recently published in *Cell Cycle*[6] describe what drives the aberrant expression of miR-155 found in CTCL.[7] They show that expression of BIC (the host gene of miR-155) is induced by the IL-2Rβγ cytokines IL-2 and IL-15 via activation of transcription factor STAT5. Apart from the cytokine-dependent expression and the direct induction by constitutive STAT5 activity as shown in this study, miR-155 has previously been described to be induced by inflammatory cytokines such as TNFα, IFNγ, or IL-1β, all of which play a key role in inflammation and cancer via STAT1 or NFkB signaling. miR-155 is just one of several major oncogenic miRNAs that are aberrantly expressed in many cancers. Several of these oncomiRs have been described to be regulated via cytokine-mediated STAT signaling. Thus, miR-21 and the miR-17–92 cluster are regulated by STAT3,[5,8] which, in turn, is activated by different cytokines, e.g., IL-6 or IL-21. Importantly, deregulated IL-6 and IL-21 signaling as well as miR-21 and miR17–92 expression has additionally been observed in different inflammatory diseases.

Through the large body of data describing miRNA function that has been produced throughout the last years we have learned that miRNAs represent an important network of fine-tuners that add an additional layer of control of gene expression. It has become very clear that aberrant miRNA expression adds a substantial contribution to the development of many different pathogenic settings. However, much still needs to be learned about the reciprocal relationship of cytokines and miRNAs in the orchestration of inflammation and disease.

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Connecting the dots in cutaneous T cell lymphoma (CTCL): STAT5 regulates malignant T cell proliferation via miR-155

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Cutaneous T-cell lymphomas (CTCLs) are a group of lymphoproliferative disorders affecting the skin. The etiology of CTCLs is unknown, and the pathogenesis remains elusive.[1] Yet CTCL provides an interesting setting for studying the link between inflammation and cancer, since lymphocytic infiltrate is the hallmark of both. Early stages of CTCL mimic benign inflammatory disorders, including psoriasis and eczema, with malignant T cells homing to the skin. This disease usually remains indolent as isolated patches and plaques for many years, but in 10–20% of cases it can progress to form tumors and/or disseminate to lymph nodes, blood and visceral organs.[1] Patients with advanced stages of CTCL often succumb to sepsis secondary to breakdown of the skin barrier function and immune suppression. Clinicians specializing in treating this cancer often face a number of important challenges. First, how to diagnose and distinguish early stages of CTCL from other benign inflammatory dermatoses? Second, how to predict which 10–20% of patients are likely to progress toward advanced stages, and, finally, how to achieve a cure of the disease with minimal toxicities? To answer these questions, better understanding of molecular CTCL carcinogenesis is urgently needed, where identified molecular players can be used as novel diagnostic/prognostic markers as well as targets for therapy.

In the article by Kopp et al., the authors establish STAT5-mediated upregulation of mir-155 as an important step in CTCL carcinogenesis.[2] Indeed, microRNA (miRNA) studies only recently became a prominent part of CTCL research. Specifically, Ralfkiaer et al. identified a set of miRNA classifiers that can be employed to distinguish early stages of CTCL from other benign inflammatory conditions.[3] Still, unfortunately, functional data on miRNA remains sparse and has only begun to emerge in the last few years. miR-155 was recently highlighted as being upregulated in CTCL.[3] This gene is a well-studied miRNA that is crucial for inflammation and is often overexpressed in various cancers. In their seminal article Kopp et al. discovered a link between miR-155 expression and JAK/STAT signaling in CTCL.[2] They provide evidence that miR-155 is induced via transcription factor STAT5 through either cytokine (IL-2/IL-15)-dependent or constitutive activation in malignant and non-malignant T cells, including PBMCs and primary CTCL cells (**Fig. 1**). Furthermore, they found miR-155 to be involved in malignant proliferation. Their results are intriguing, because they connect some of the major hallmarks in CTCL: an increased expression of oncomiR-155, deregulation of JAK/STAT signaling pathways, and a persistent activation of STAT transcription factors.[2,4]

While aberrant activation of multiple STAT proteins has been observed in various cancers, until recently, CTCL research has primarily focused on STAT3 as the major culprit in the effects of aberrant JAK/STAT signaling.[5] Yet several studies have also implicated STAT5 as being aberrantly activated in malignant T cells. However, little was known about downstream targets and cellular consequences of STAT5 activation in CTCL. Now Kopp et al. document that this well-described oncomiR, miR-155, is a novel downstream target of STAT5 and is involved in malignant proliferation of T cells.[2] Since miR-155 has also been implicated in genomic instability in cancer, it is possible that STAT5, via induction of miR-155, also drives genomic instability, a key feature of CTCL.

As mentioned above, one of the major obstacles in managing CTCL is our inability to consistently achieve cure of this cancer. Due to its heterogeneity, there is no common genetic aberration or biomarker providing a reliable therapeutic target for patients. To achieve effective cure, CTCL therapy is in need of new targets and treatment strategies. Kopp et al. showed that treatment of malignant cells with JAK inhibitor tofacitinib (CP 690 550) strongly inhibits miR-155 expression and STAT5 activation. These results suggest a therapeutic potential of JAK inhibitors. Tofacitinib

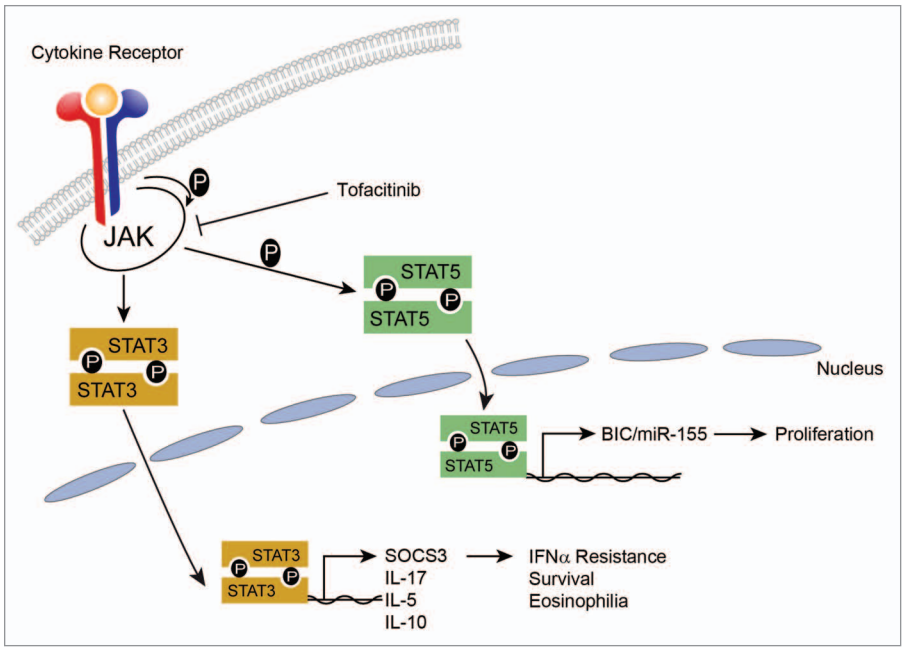

Figure 1. STAT5 signaling trans activates miR-155 expression, which can be blocked upstream at the level of JAK kinase signaling by tofacitinib inhibitor.

is already clinically approved for treatment of rheumatoid arthritis and is now being tested in clinical trials for psoriasis.[6] It would be very interesting to evaluate the potential of tofacitinib in combination with already existing therapies for CTCL. In summary, these combined results hold great potential for diagnosis and treatment of CTCL.

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3D culture adds an extra dimension to targeted epigenetic therapies

Comment on: Amatangelo MD, et al. Cell Cycle 2013; 12:2113–9; PMID:23759589; http://dx.doi.org/10.4161/cc.25163

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Histone methyltransferases (HMT) are chromatin modifiers that regulate the transcriptomic landscape in normal development as well as in diseases such as cancer.[1] Enhancer of Zeste 2 (EZH2), a component of the polycomb repressive complex 2 (PRC2), trimethylates histone 3 lysine 27 (H3K27me3), resulting in a more compact chromatin structure known to repress gene activation.[2] Overexpression of EZH2 has been strongly implicated in oncogenesis of many different cancers, including ovarian cancer (OC),[2] and increased EZH2 activity has been linked to OC resistance to platinum-based chemotherapy, presumably by inhibiting crucial tumor suppressors and genes involved in OC metastasis such as integrins.[2,3] Although HMT inhibitors (HMTI) that target EZH2 specifically or HMTs in general are promising anticancer therapeutics,[1] the mechanism(s) underlying EZH2 regulation is incompletely understood, and the use of clinically relevant model systems to better understand the translational potential of this important class of epigenetic modifiers is clearly an important area of investigation.

In the July 1, 2013 issue of *Cell Cycle*, Amatangelo and colleagues investigated the effect of a specific inhibitor of EZH2, GSK343, on human epithelial ovarian cancer.[4] After GSK343 treatment of ovarian cancer (OC) cells grown on a 2D monolayer, a 90% decrease in total H3K27me3 was observed, demonstrating drug specificity to EZH2, and EZH2 protein levels remained unchanged, corroborating the mechanism of GSK343 action as an inhibitor of EZH2 HMT activity. Despite this, no effect on growth was observed on OC cells in 2D culture, although previous studies have reported that reducing the HMT activity of EZH2 markedly alters cell physiology in OC and other cancers. For example, phosphorylation of EZH2 by protein kinase B (AKT) on serine 21 (S21) reduced EZH2 enzymatic activity and as well as integrin α2 expression.[5] In addition, EZH2 phosphorylation on residues threonine 350 and 487 (T350 and T487) by cyclin-dependent kinase 1 and 2 (CDK1 and CDK2), respectively, inhibited cell proliferation, migration, and anchorage-independent growth, indicating a role for EZH2 HMT activity in migration and invasion.[6,7]

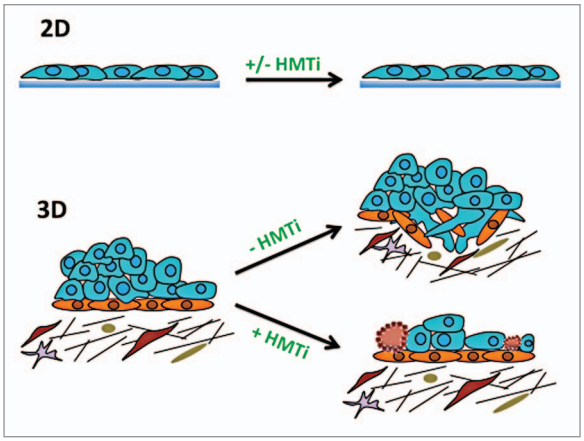

Figure 1. Comparing the sensitivity of epithelial ovarian cancer cells to the histone methyltransferase inhibitor (HMTI) GSK343 in 2D (i.e., plastic, upper) and 3D (i.e., matrigel extracellular matrix, lower). No effect of GSK343 on ovarian cancer cell migration, invasion, or apoptosis was observed using 2D culture conditions, despite the fact that the drug significantly reduced H3K27me3 levels. In 3D culture, however, GSK343 significantly inhibited ovarian cancer cell invasion and reduced cell survival, which was correlated with apoptosis induction (indicated by brown cells). The third dimension showcased the association of altered pathways (possibly by dysregulation of cellular proteins involved in ECM communication) with decreased in EZH2 activity.

To further investigate the effect of GSK343-mediated EZH2 inhibition on OC, Amatangelo and colleagues[4] extended the scope of their study by using a 3D cell culture system composed of a matrigel extracellular matrix (ECM). It is well known that the tumor microenvironment is both heterogeneous in nature, composed stromal fibroblasts, immune cells, and vascular endothelial cells, and can significantly impact both the metastatic potential of cancer cells as well as their ability to resist chemotherapy. For these reasons, the ECM closely recapitulates the tumor microenvironment, representing a more clinically relevant model system compared with monolayer culture conditions on plastic. Taking this approach, Amatangelo and colleagues observed that

GSK343 treatment inhibited OC cell growth and invasion in a 3D culture and correlated with apoptosis induction in OC cells.[4] The results indicate regulation of EZH2 by the OC-ECM interaction as well as improved efficacy of GSK343 to suppress the ability of OC to remodel the ECM and metastasize (**Fig. 1**).

As epigenetic therapies for OC are in the clinical arena[8] (and see SGI-110 in Combination With Carboplatin in Ovarian Identifier: NCT01696032, CancerClinicalTrials.gov), the exciting new study by the Zhang lab provides compelling evidence for using 3D culture systems to gain key insight into the biological roles of EHZ2 in OC and the sensitivity of this cancer to EZH2-specific inhibitors. Furthermore, as suggested by the Zhang lab,[4] the numerous discrepancies observed between the efficacy of inhibitors in 2D culture and in vivo animal systems may be better explained by using the third dimension.

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