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Cell Cycle News & Views

Hot and toxic: Hyperthermia and anti-mitotic drugs in cancer therapy

Comment on: Giovinazzi S, et al. Cell Cycle 2013; 12;2598–607
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The main feature of tumor cells is their ability to divide unrestrictedly. Cellular division critically depends on successful mitosis. Thus, perhaps unsurprisingly, among the most powerful anticancer drugs are the ones that target mitosis. Proper segregation of chromosomes during mitosis is ensured by a highly coordinated and dynamic process of polymerization/depolymerization of mitotic spindle microtubules. Hence, natural microtubule-poisoning drugs, such as Paclitaxel (PTX or Taxol) and vinblastine are commonly used as anti-neoplastic agents for the treatment of solid tumors, including breast, ovarian, lung, and head and neck malignancies.[1] The consequence of treatment with these drugs is mitotic arrest, which, in turn, activates the spindle assembly checkpoint (SAC). Tumor cells employ several mechanisms to skip SAC, including ubiquitin-dependent degradation/inactivation of the cyclin B/Cdk1 complex by the anaphase-promoting complex/cyclosome (APC/C). The latter is an E3 ubiquitin ligase that operates via 26S proteasome[2] and is positively regulated by cyclin B/Cdk1-mediated phosphorylation. Inactivation of Cdk1 results in attenuation of the APC/C activity, which, in turn, is required for degradation of securin and the release of sister chromatids, thus promoting the metaphase-to-anaphase transition. Another mechanism of slippage from SAC is attenuation of mitotic kinase Aurora A, which is necessary for the proper separation of centrosomes after the mitotic spindle has been formed.[3] However, for escaping SAC, cancer cells pay their toll, i.e., they die because of mitotic catastrophe.[4]

Unfortunately, during the prolonged anti-mitotic therapy, tumor cells acquire resistance to PTX by overexpressing mutated versions of tubulin proteins inert to PTX, or by modulating the expression of micro-RNAs that target the Bcl-2 family of proteins. Therefore, the problem of overcoming PTX resistance urgently requires its solution and warrants extensive studies in this area. The idea of combining physiological (39.5–45 °C) hyperthermia (HT) with cancer therapy is currently being actively explored in clinical studies. For example, several phase III trials comparing radiotherapy alone or with hyperthermia have shown a beneficial effect of hyperthermia in terms of local control of recurrent breast cancer and malignant melanoma and survival (e.g., head and neck lymph-node metastases, glioblastoma, cervical carcinoma, etc.).[5]

In this respect, the study by Giovinazzi et al. published in the present issue of *Cell Cycle* is timely and interesting. The authors showed that hyperthermia (HT) and APC/C inhibition have opposite effects on the fate of tumor cells arrested in mitosis; HT provoked mitotic slippage of breast tumor cells, resulting in mitotic catastrophe, whereas the inhibitor of APC/C, proTAME, on the contrary, prolonged the time of mitosis and led to apoptosis. Importantly, these effects were independent of the means by which cells were arrested in mitosis, i.e., either by treatment with Aurora A inhibitor MLN8054 or with Paclitaxel (PTX). On the therapeutic side, the authors showed that HT enhanced the cytotoxic effect of PTX on breast cancer cells irrespective of their status toward sensitivity to PTX.

How HT promotes the escape of PTX-arrested cells is yet not clear. In general, HT was reported to disrupt the microtubule network. However, in the case of prior PTX treatment, the microtubule bundles remained intact.[6] The most apparent effect of HT was observed at the level of centrosomes. In this respect, members of the heat shock proteins family (HSPs) play the pivotal role in assisting repair of centrosomes after heat shock. Microinjection of Hsp73 antibodies retarded recovery of the interphase centrosome structure and microtubule re-assembly after HT, whereas injection of purified Hsp73 before heat shock enhanced these processes.[7] Collectively, these findings suggest an intriguing possibility that the combination of chemical inhibitors of HSPs (17-AAG and KNK437, or quercetin)[8] with PTX and HT will facilitate the slippage of cancer cells from mitosis and, hence, augment the antitumor effect of combination therapy, especially in the case of PTX-resistant malignancies.

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Carbonic anhydrase 9 (CA9) and redox signaling in cancer-associated fibroblasts: Therapeutic implications

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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Recent advances in cancer research have focused on the additional dimension of complexity within solid tumors that constitutes the tumor microenvironment. The tumor microenvironment is comprised of a complex network of cancer cells and secondary stromal cells, which includes endothelial cells, inflammatory cells and cancer-associated fibroblasts (CAFs), and is influenced by environmental factors including nutrient availability and hypoxia.

It is becoming increasingly apparent that intratumoral communication between cancer cells and the associated cells of the microenvironment is vital to tumor growth and malignant progression. Furthermore, this cross-talk is not merely mediated by cytokines and growth factors, but may extend to the metabolic reprogramming of both cancer cells and CAFs. In this model, fibroblasts are converted by cancer cells to energy-producing powerhouses, inducing an increased glycolytic rate and autophagy, mediated by enhanced oxidative stress and the redox stabilization of hypoxia-inducible factor-1 (HIF-1).[1] This metabolic reprogramming of CAFs not only leads to the production of high-energy metabolites fueling epithelial cancer cells and subsequent tumor growth, but also increases the acidification of the tumor microenvironment via the production and export of acidic metabolites, including lactic and carbonic acids (**Fig. 1**). In this way, CAFs act to promote a metastatic phenotype in two ways, first via the direct acidification of the microenvironment, accelerating the degradation of the extracellular matrix, promoting invasion, and second by simulating a proinflammatory environment, indirectly promoting the EMT program in epithelial cancer cells.

For these reasons, this cross-talk between tumor and stromal cells is thought to play an active role in acquiring the capacity for invasion and metastasis. The contribution of pH regulators to the metastatic phenotype has been intensely studied in epithelial cancer cells and has largely focused on the carbonic anhydrase (CA) family of enzymes that catalyze the hydration of carbon dioxide to biocarbonate and protons. Of note is the HIF-1-regulated CAIX, which mediates the acidification of the tumor microenvironment and enhances tumor growth and migration of tumor cells.[2] The expression of tumor-associated CAIX in clinical samples is reported to be an independent prognostic factor associated with both poor prognosis and increased incidence of metastasis in many tumor types, and hence may act as a surrogate biomarker of hypoxia.[3] Moreover, recent studies have reported an association between the level of soluble plasma CAIX and reduced survival, and radiolabelled antibodies targeting CAIX have been developed as imaging tools for detecting tumor hypoxia in the clinic.[4]

The recent paper by Fiaschi and colleagues has reported, for the first time, the role of CAIX in a novel mechanism by which CAFs promote metastasis.[5] They observed that tumor cells stimulated the de novo expression of CAIX, resulting in extracellular acidification in CAFs in vitro and in CAFs isolated from patient samples of prostate carcinoma, stressing the clinical relevance of this finding. The expression of CAIX in CAFs was associated with ROS-dependent stabilization of HIF-1 in normoxia, and, as such, fits perfectly with previous reports of redox stabilization of HIF-1 in normoxia involved in metabolic rewiring in both prostate and breast cancer.[1,6] Functionally, upregulation of CAIX in CAFs resulted in an increase in MMP2 and MMP9 activity, with decreased E-cadherin expression and increased tumor cell invasion, suggestive of activation of EMT in epithelial cancer cells (**Fig. 1**). Silencing of CAIX in CAFs was sufficient to prevent spontaneous lung metastasis in vivo when co-injected with prostate cancer cells, confirming that CAF-associated CAIX is essential for EMT and metastasis in vivo.

The limited tissue distribution of CAIX in normal tissue makes it an especially attractive target for cancer therapy. Several inhibitors and antibodies targeting CAIX are currently in clinical development and have entered clinical trial,[7,8] and, taken together, these findings suggest that CAIX may not only be a novel marker of CAFs, but also an important novel therapeutic strategy for targeting both CAF- as well as hypoxia-driven EMT.

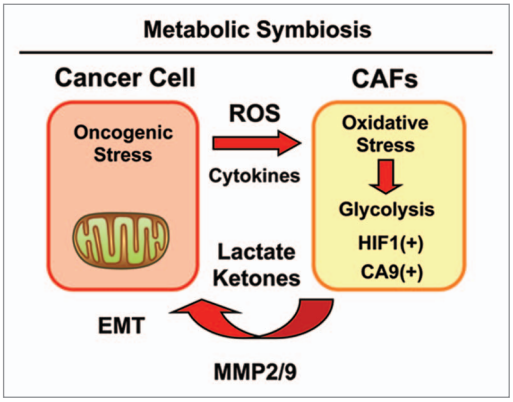

Figure 1. The role of CAFs, CA9, and metabolic symbiosis in promoting the EMT in epithelial cancer cells.

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Identifying the stromal cell type that contributes to tumor aggressiveness associated with carbonic anhydrase IX

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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Otto Warburg was the first to postulate a role for cell metabolism in carcinogenesis. Hanahan and Weinberg recently updated their seminal review to include metabolic reprogramming as a hallmark of cancer.[1] While normal cells predominantly depend on mitochondrial oxidative phosphorylation for their energy needs, cancer cells favor aerobic glycolysis, also known as the Warburg effect. This unique metabolic shift provides a survival advantage to the cancer cells in the developing tumor microenvironment and, paradoxically, provides oncologists with potential therapeutic targets. Indeed, metabolic changes have been described as the "Achilles' heel" of cancer.[2] One such metabolic change is the acidification of the tumor microenvironment by carbonic anhydrases (CAs), especially CAIX. CAIX expression is regulated by the pro-survival transcription factor hypoxia-inducible factor-1α (HIF-1α). CAIX is overexpressed in many tumor types and has been linked to poor prognosis, purportedly due to its involvement in the breakdown of extracellular matrix, protease, and growth factor activation and augmentation of metastatic potential. Previous research has focused predominantly on the metabolic and molecular features of tumor cells, but there is an increasing awareness that stromal cells recruited to the tumor microenvironment are important contributors to the development, progression, and aggressiveness of tumors.

In the June 1, 2013 issue of *Cell Cycle*, Chiarugi and colleagues demonstrated the role of CAIX-expressing cancer-associated fibroblasts (CAFs) in regulating the epithelial–mesenchymal transition (EMT) of prostate cancer cells.[3] They report that normal human prostate fibroblasts (HPFs) do not express CAIX; however, exposing HPF to conditioned media (CM) from prostate cancer (PCa) cells activates HPF cells to CAFs. CAIX expression was also induced in prostate cancer (PCa) cells treated with CM from CAFs, highlighting the cross-talk between the tumor and its microenvironment (**Fig. 1**). Interestingly, CAIX was expressed at similar levels in CAFs and serum-starved PCa cells, but PCa cells treated with CM from CAFs expressed higher CAIX levels than CAFs themselves. However, CAIX activity was higher in CAFs compared with PCa cells treated with CAF CM. CAIX

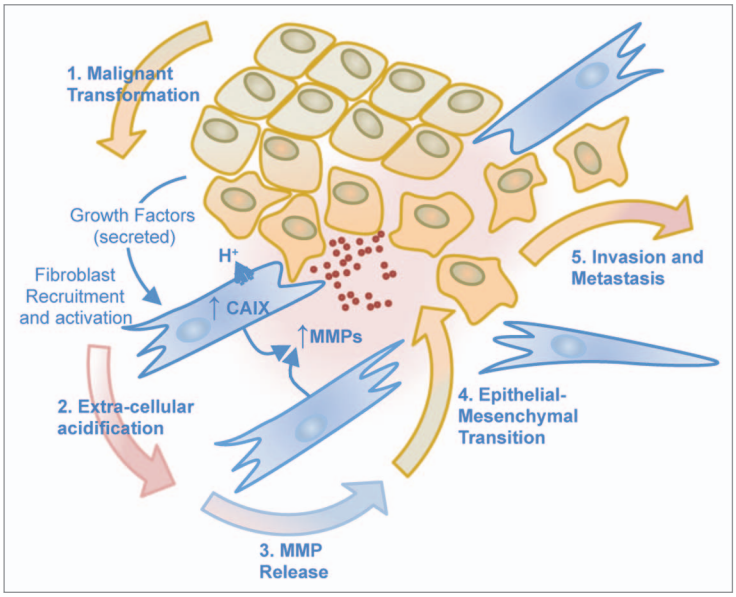

Figure 1. Tumor microenvironmental cross-talk mediates epithelial–mesenchymal transition. (1) Malignant transformation induces the secretion of growth factors; these growth factors activate resident and recruited fibroblasts to CAFs and increase CAIX expression in the tumor microenvironment. (2) Increased expression and activity of CAIX causes extracellular acidification. (3) Extracellular acidification induces MMP secretion. (4) MMP secretion degrades the extracellular matrix and drives epithelial–mesenchymal transition leading to (5) invasion and metastasis.

expression in both PCa cells and CAFs was HIF-1α-dependent despite these experiments being conducted under normoxic conditions; this observation further supports that the activation of HIF1α signaling was mediated by redox-based stabilization of HIF1α.[4] CAIX inhibition decreased extracellular acidification thereby demonstrating that CAIX is necessary and sufficient for such acidification.

The role of matrix metalloproteinases (MMPs) in aggressive/metastatic disease and their response to low pH are well documented.[5] Consequently, the authors investigated the link between CAIX and MMP expression. CAIX-induced acidosis increased the expression of MMPs in CAFs, and inhibition of CAIX decreased the secretion of MMP-2 and MMP-9. Inhibition of MMPs reduced the invasiveness of PCa cells. Addition of recombinant MMPs to CAIX inhibited CM rescues ability of PCA cells to undergo EMT. In immune-compromised mice, inhibition of CAIX in CAFs reduced the ability of PCa cells to form viable tumors and effectively metastasise to the lung.

The cellular and mechanistic insights provided by this article are exciting and timely, but it is important that these insights be applied in patient samples to understand the clinical significance of the findings. We have previously reported, in 2 independent head and neck cancer cohorts, that stromal CAIX levels are more strongly associated with poor survival than tumor CAIX.[6,7] High-stromal CAIX was also associated with increased nodal metastasis.[7] However, we did not identify the specific contributing stromal cell-types. In the future, co-staining tissue micro-arrays with α-smooth muscle actin (a specific marker for CAFs) would potentially improve the definition of the stromal contribution to CAIX expression and association with prognosis. Chiarugi and colleagues report CAFs as the main protagonists in the CAIX-induced tumor aggressiveness, but the role of other cell types in the tumor microenvironment should be investigated. Furthermore, the direct effect of CAIX inhibition in PCa cells needs to be determined.

CAIX is an attractive therapeutic target, because its expression is relatively tumor specific, several low-toxicity pharmaceuticals are available, and novel analogs of existing inhibitors are currently being tested.[8] Given the disappointing results of MMP inhibition trials,[5] targeted reduction of MMP2 and MMP9 by inhibition of CAIX may provide an alternative strategy. Also, the effects of CAIX inhibition on other MMPs and the potential for regulation by other compensatory mechanisms should be addressed.

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DUBs "found in translation": USP15 controls stability of newly synthesized REST

Comment on: Faronato M, et al. Cell Cycle 2013; 12:1964–77; PMID:23708518; http://dx.doi.org/10.4161/cc.25035

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Protein ubiquitination is a versatile covalent modification that controls myriad biological processes. In an analogy to kinases and phosphatases, E3 ligases and de-ubiquitinating enzymes (DUBs) provide a dynamic equilibrium for substrate modification. While it's estimated that over 600 E3 ligase complexes exist in human cells, ≈90 DUBs control the removal of ubiquitin from substrates. A well-established, but clearly not the sole, role for protein ubiquitination is to create a signal for targeting substrates for 26S proteasomal degradation. This function is part of protein homeostasis to balance protein synthesis, to terminate protein function at defined stages of the cell cycle or cellular conditions, or to eliminate misfolded proteins upon their synthesis. Early and more recent studies indicated that up to 30% of newly synthesized proteins are rapidly ubiquitinated and degraded.[1-3] Indeed, E3 ligases such as Ltn1 and Hel2 associate with ribosomes and act as quality-control factors for newly synthesized proteins.[2,4] The simplest explanation for this phenomenon is that newly synthesized proteins are prone to misfolding and have to be rapidly eliminated. However, even if it was predicted, no DUBs had been reported to act on ribosomes.

In the study by Faronato et al., a DUB is now reported to control protein stability of newly synthesized proteins.[5] In an unbiased DUB siRNA screen, USP15 was found as a regulator of the stability of the RE1 silencing transcription factor (REST). REST is a key transcriptional repressor that silences the expression of neuronal genes in non-neuronal cells. Targeting REST for ubiquitin-mediated proteasomal degradation is critical both for neuronal differentiation and for activation of the spindle checkpoint and mitotic exit. Phosphorylation of REST allows its recognition by the β-TrCP E3 ligase, which promotes REST ubiquitination and proteasomal degradation.[6,7] Therefore, USP15 could control REST stability by opposing β-TrCP function. However, 2 key observations prompted the authors to investigate alternative mechanisms of REST stability control by USP15. As a transcriptional repressor REST is mainly found in the nucleus, O-glycosylated, with apparent molecular weight of 180–220 kDa, whereas the non-glycosylated newly synthesized form migrates as a 120 kDa protein. While USP15 controls the levels of both nuclear and cytoplasmic REST, it is mainly localized in the cytoplasm. The other key observation was that in conditions where translation was inhibited,

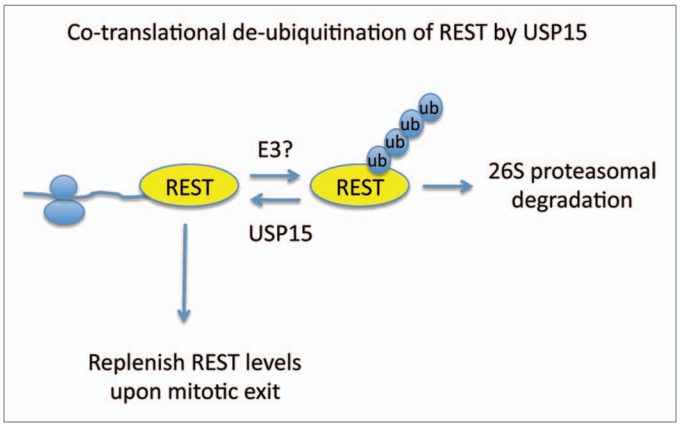

Figure 1. USP15 opposes the function of an unknown E3 ligase to stabilize newly synthesized REST protein. As REST is degraded by the β-TrCP E3 ligase at mitosis, USP15 function is critical to replenish REST levels for the new cell cycle.

USP15 knockdown failed to accelerate REST degradation. Therefore, the authors tested the hypothesis that USP15 controls the turnover of newly synthesized REST. Experiments addressing the re-synthesis of REST after removal of translational block showed that USP15 controls the stability of newly synthesized REST protein. Critically, ribosome-profiling experiments indicated the presence of USP15 in polysome-containing fractions. But what is the biological consequence for such regulation? As already mentioned β-TrCP-mediated degradation of REST occurs predominantly at mitosis. While USP15 does not antagonize β-TrCP-mediated REST degradation, is required for the rapid replenishment of REST upon mitotic exit for the beginning of the new cell cycle. These observations provide evidence for a co-translational role of USP15 in REST stability control through de-ubiquitination (**Fig. 1**). The model is further supported from the observations that inhibition of the 26S proteasome could rescue REST stability both in steady-state conditions and during translational recovery. It will be interesting to determine whether USP15 is a ribosome-associated factor or directly interacts with co-translated REST. The dynamic and reversible mode of function for protein ubiquitination predicts the presence of an E3 ligase operating along USP15. The studies by Faronato et al. also indicate that the function of the ubiquitin-proteasome pathway at the ribosome may not be restricted as a quality control mechanism to eliminate misfolded proteins, but also to provide a rapidly responding system to replenish factors with key roles in cell cycle.

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