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Cell Cycle News & Views

Tumor-stroma relationships: Who's the driver?

Comment on: Witkiewicz, et al. Cell Cycle 2012; 11:1108–17; PMID:22313602; http://dx.doi.org/10.4161/cc.11.6.19530

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Cancer is characterized by somatic mutations that provide a growth and survival advantage. The mutations are a function of chance because of the random nature of mutagenesis. However, the biology in the tumor cells drives the selection of mutations that are advantageous to the cancer.[1] In addition, the tumor environment exerts a selective pressure on the tumor. For example, the hypoxic environment of a tumor induces HIF, stimulating VEGF production, which signals to the non-tumor endothelial cells to stimulate angiogenesis. Witkiewicz et al. have assessed the association of another non-tumor marker, MCT4, in triple-negative breast cancer (TNBC).[2]

Triple-negative breast cancers are estrogen and progesterone receptor-negative and HER-2-negative and account for 10–20% of all breast cancers.[3] Standard treatment is surgery with radiotherapy and adjuvant chemotherapy, sometimes with biologic agents. Although TNBCs are generally very susceptible to chemotherapy, they are often associated with a shorter median time to relapse and early death. One important goal is to identify prognostic biomarkers to reliably select high- and low-risk subsets of TNBC.

Prognostic biomarkers do not have to be limited to the tumor cell. The ratio of tumor to stroma in TNBC is a predictor of outcome; tumors with < 50% stroma have a 5-year progression-free survival and overall survival of 85% and 89% compared with 45% and 65% in tumors with > 50% stroma.[4] Does the biology of the stroma predict response in TNBC? Loss of stromal caveolin-1 (Cav-1) is predictive in TNBCs; 75.5% of patients are alive at 5 y with high stromal Cav-1 compared with 9.4% of patients with low stromal Cav-1.[5] There is some controversy; not all data supports the prognostic association, with arguments that Cav-1 is a tumor suppressor in some situations and an oncogene in other settings.[6] Loss of Cav-1 increases the levels of enzymes in glycolysis and lactate dehydrogenase. Hence, the intracellular levels of lactate could be elevated in Cav-1-null stromal cells, and the level of the lactate transporter MCT4 could be increased to maintain intracellular pH.[7] An inverse correlation between Cav-1 and MCT4 expression is a plausible hypothesis. Witkiewicz et al. showed high stromal MCT4 predicts for poor outcome in TNBC.[2] Combining the data for both Cav-1 and MCT4 improved the prognostic power of the data, certainly for the intermediate risk groups.

MCT4 is a plausible therapeutic target whether considering excessive production of lactate by stromal tissue or tumor cells. In either scenario, the metabolism of the tumor would be disturbed, causing intracellular acidosis if MCT4 is on the tumor cells and starving the tumor if MCT4 is on the stromal cells. Would patients with TNBC with Cav-1 low/MCT4 high stromal tissue elicit a clinical benefit from decreasing the availability of lactate? Possibly. However, the tumor metabolism might adapt to use alternative sources of carbon. On the upside, the acidosis in the MCT4-positive stromal cells might kill the stromal cells, lowering the stroma/tumor ratio and subsequently extending relapse-free survival.

The characterization of the cancer-stromal relationship is gaining momentum, from direct interactions, e.g., through integrins, to signaling molecules such as VEGF.[8] Metabolically, chronic lymphocytic leukemia cells can modulate their redox status through the synthesis of GSH from cysteine that is released by bone marrow stromal cells.[9] The bone marrow cells are not bystanders but import cystine and convert it to cysteine that is exported to the microenvironment.

In conclusion, there is crosstalk at multiple levels between tumor cells and the stroma, providing a rationale to target the stroma. This is unlikely to be a panacea for treating cancer as shown by the inter- and intra-tumor-type heterogeneity that has been shown for tumor-stroma interactions. What will predict which tumors will respond to stroma directed therapies; the biology of the stroma, the biology and genetics of the tumor or both? As the volume of cancer genome data grows, we would be wise to consider how the genetics of tumors is molded by the microenvironment and vice versa, and how we might target the microenvironment for clinical benefit.

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Peptidyl-prolyl isomerization for cytokinetic abscission: Fold and cut

Comment on: Bannon JH, et al. Cell Cycle 2012; 11:1340–53; PMID: 22421161; http://dx.doi.org/10.4161/cc.19711

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Cytokinesis is the final stage of cell division, during which physical separation of the two daughter cells is accomplished. It starts with ingression of the plasma membrane mediated by an actomyosin contractile ring leading to the formation of a cleavage furrow. By the end of furrowing, the dividing cells are connected by an intercellular microtubule-loaded bridge containing the midbody. During the final phase of cytokinesis, in a process called abscission, this bridge is severed, and two daughter cells are formed. Cells that have problems at this stage maintain the intercellular bridge for a prolonged period of time, and if the bridge cannot be severed, cells become polyploid. Polyploidy has been shown to promote tumor development.[1]

Central players in abscission are the members of the ESCRT (endosomal sorting complex required for transport) machinery, which constrict the intercellular bridge to allow its fission.[2] These factors are recruited to the site of abscission by a protein named CEP55.[3] CEP55's function, in turn, is regulated by phosphorylation, and the phosphorylation-dependent peptidyl-prolyl isomerase Pin1.[4] Pin1 is a member of the parvulin family of prolyl isomerases that catalyze *cis-trans* isomerization of proline amide bonds. Another family of peptidyl-prolyl isomerases are the cyclophilins. Generally, these isomerases control the folding/conformation of proteins.

In a new study, Bannon et al. reported their analysis of the whereabouts and functions of one of the cyclophilins, cyclophilin A (CypA, also called peptidyl-prolyl isomerase A) during cell division.[5] They demonstrate that CypA is a centrosomal protein during interphase and mitosis, and that it translocates to the midbody during cytokinesis. Although CypA is centrosomal, CypA deficiency does not cause any detectable mitotic abnormalities. Bipolar spindle formation, the spindle assembly/mitotic checkpoint and the time taken from anaphase until furrow ingression in telophase are all comparable in CypA-knockout and control cells, indicating that its function at the centrosome, if any, is at least redundant. However, knockdown or knockout of CypA does cause a defect at a later stage, namely during cytokinesis. More specifically, cells lacking CypA remained connected by a thin cytoplasmic intercellular bridge for a prolonged period of time and displayed a higher number of polyploid cells as compared with control cells. Moreover, CypA-deficient cells exhibit supernumerary centrosomes and decreased proliferation. By using a mutant of CypA (R55A) that has compromised isomerase activity, the authors showed that isomerization activity of CypA is not required for its localization to the intercellular bridge but is essential for successful completion of cytokinesis.

An interesting area for further studies may include the identification of substrates of CypA that control cytokinesis. To this end, database mining for the CypA consensus binding motif FGPXLP could be informative.[6] In this motif, only the amino acids FGP are very strict. Another, complementary, approach to identify CypA substrates would be to search for interaction partners of CypA that are known to play a role in cytokinesis. Notably, cells that have reduced activity of calcineurin, a heterodimeric protein phosphatase that interacts with CypA and does contain a CypA consensus binding motif, display cytokinesis defects that are similar to those seen in CypA-knockdown cells.[7,8] Moreover, cyclosporine A (CsA), a fungal immunosuppressive compound that can inhibit both calcineurin and CypA, induces a cytokinesis defect.[8] Interestingly, CypA may stimulate calcineurin activity.[7] Whether this is dependent on isomerase activity remains an open question. Nevertheless, it is tempting to speculate that CypA may regulate cytokinesis through activation of calcineurin.

Peptidyl-prolyl isomerization plays a crucial role in a plethora of cellular processes, and the results of Bannon et al. together with previously published data suggest that at least two isomerases, CypA and Pin1, regulate cytokinetic abscission.[4,5] Interestingly, in yeast, the loss of ESS1 (yeast Pin1) can be complemented by enhanced expression of CPH1 (CypA), suggesting that Pin1 substrates can be isomerised by CypA[9]. However, it appears that in mammalian cells, they are not acting redundantly, as knockdown/knockout of each individually (Pin1 or CypA) results in a cytokinesis defect.[4]

CypA is overexpressed in multiple cancers, and it has been hypothesized that tumor cells exploit protein isomerization to promote their growth. Indeed, CypA deficiency does affect cell division and thus may be used as a therapeutic target to limit the proliferation of tumor cells.

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A new tool for an old problem: Synchronizing fission yeast cells during meiosis using an ATP analog-sensitive protein kinase

Comment on: Cipak L, et al. Cell Cycle 2012; 11:1625–32; PMID:22487684
and Guerra-Moreno A, et al. Cell Cycle 2012; 11:1620–4; PMID:22456336

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Meiosis represents a fundamental process involved in the formation of haploid gametes from diploid cells during sexual reproduction of eukaryotic organisms. It was discovered in the second half of 19th century and, after pioneering studies of Thomas H. Morgan in the 1910s, has become a hot topic of countless genetic studies. In spite of more than a century's worth of research on meiosis, many of its crucial features remain unknown. For example, one of the highlights of first meiotic division (meiosis I) is resistance of centromeric cohesion to cleavage by separase. Although it is clear that this is an essential prerequisite for successful segregation of homologous chromosomes, its molecular nature is not fully understood.[1] A model organism particularly suitable for investigating meiosis-related molecular mechanisms is fission yeast *Schizosaccharomyces pombe*. Vegetative diploid cells of this yeast readily undergo meiotic program when starved for nitrogen and (unlike budding yeasts) produce ordered tetrads that allow direct determination of segregation during the first and second meiotic divisions. However, as investigations of events accompanying meiosis require highly synchronous cell populations, naturally sporulating cells exhibiting a low level of synchrony are not suitable for systematic analyses. For more than two decades, these studies relied on the use of thermosensitive *pat1–114* allele. The wild-type *pat1*$^{+}$ gene encodes protein kinase Pat1/Ran1, which negatively controls the initiation of meiosis.[2-4] Upon induction at non-permissive temperature, nitrogen-starving *pat1–114/pat1–114* cells progress through the meiotic program in a highly synchronous manner. Although the meiosis in the *pat1–114* mutant is similar to that of wild-type cells, non-permissive temperature itself has adverse effects. For example, the cells cultivated at elevated temperature exhibit aberrant centromere positioning, chromosome missegregation, substantial reduction of recombination and poor viability of spores (**Fig. 1**). Therefore, synchronizing meiotic cells at physiological temperature is highly desired. To address this problem, Cipak et al.[5] and Guerra-Moreno et al.[7] took advantage of chemical genetics. They used an approach based on conditional inactivation of target protein kinase by cell-permeable ATP analogs (1-NM-PP1 and 3-MB-PP1).[6,8,9] Cipak et al.[5] constructed the *pat1-as2* (ATP analog-sensitive) allele coding for the Pat1 kinase with altered ATP-binding pocket. The mutant kinase Pat1(L95A) can be easily inactivated by addition 1-NM-PP1 to cultivation media, enabling the induction of the meiotic program at physiological temperature. As expected, the level of synchrony in the *pat1-as2* population is similar to *pat1–114* cells cultivated at non-permissive temperature. Importantly, the use of ATP analog-sensitive alleles eliminates some of the abnormalities observed at elevated temperature. Moreover, the authors further improved their experimental system by combining the *pat1-as2* allele with activating pheromone signaling by ectopic expression of both mating-type loci. This improved fidelity of chromosome segregation, spore viability as well as recombination to levels similar to that observed in the wild-type cells (**Fig. 1**). Guerra-Moreno et al.[7] synchronized meiosis using the Pat1(L95G) mutant that can be inactivated by 3-MB-PP1. Moreover, they found that induced cells exhibit similar meiosis-related transcription events as the *pat1–114* strain (except for the transcription pattern of the stress response

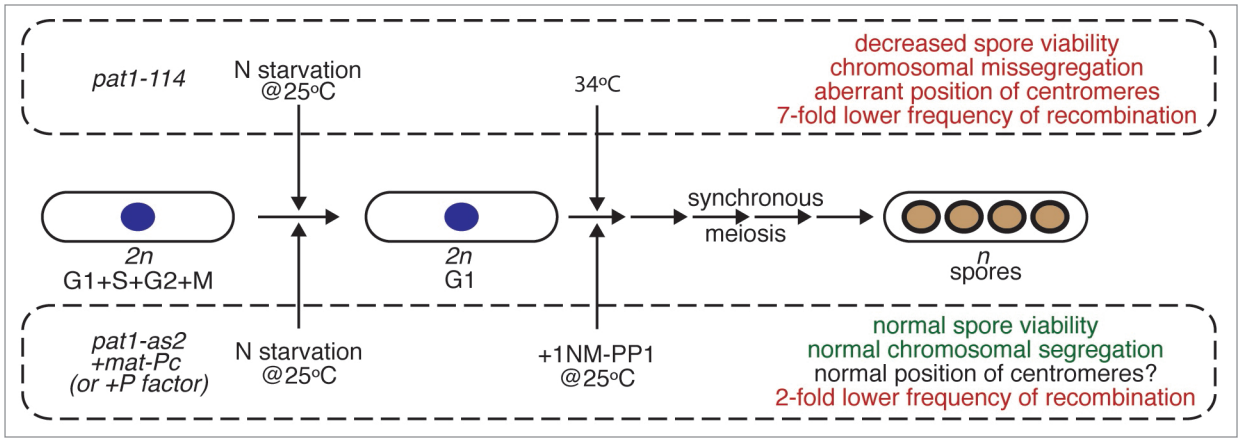

Figure 1. Induction of synchronous meiotic cultures at physiological temperature. The standard procedure[4] for obtaining synchronous meiotic cultures employs a temperature-sensitive allele (*pat1–114*) of the negative regulator of meiosis, protein kinase Pat1. However, due to the increased temperature the cells undergoing meiosis exhibit several abnormalities (in red). Cipak et al.[5,6] prepared an ATP analog (1-NM-PP1) sensitive version of Pat1 [Pat1(L95A)] that can be inhibited at physiological temperature. When Pat1(L95A) carrying strain also contained ectopically expressed *mat-Pc* the authors observed a substantial improvement in most of the analyzed characteristics of meiosis (in green). In a similar study, Guerra-Moreno et al.[7] synchronized meiotic cells by inactivating of Pat1(L95G) mutant by another ATP analog (3-MB-PP1).

gene *hsp9* probably due to cultivation of the *pat1–114* mutant at elevated temperature).

In summary, both reports[5,7] further strengthened the advantages of fission yeast as the workhorse in cell biology. The experimental tool they developed for synchronization of entire cell populations at nearly physiological conditions provides a promising venue for upcoming discoveries in the field of meiosis.

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CHEKing out of mitosis

Comment on: Wilsker D, et al. Cell Cycle 2012; 11:1564–72; PMID:22433954; http://dx.doi.org/10.4161/cc.19944

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Genomic instability and accumulation of mutations is one of the features of cancer. In order to preserve genome integrity, cells require several evolutionary conserved processes, such as the DNA damage response and cell cycle checkpoints.[1] In fact, the DNA damage response has been proposed to constitute an earlier barrier to tumorigenesis.[2,3] However, the mechanism(s) by which cells maintain genome integrity, in particular a diploid complement, remain poorly understood.

In a previous issue of *Cell Cycle*, Bunz and colleagues set out to investigate the role of Chk1 in the preservation of stable ploidy.[4] Using isogenic colorectal cancer cell lines that differ in p53 status, the authors generated cell lines hemizygous for *CHK1* (homozygous *CHK1*-null cells are not viable) and show that *CHK1* haploinsufficiency in a p53-deficient background leads to the appearance of a tetraploid subpopulation.[4] Interestingly, *CHK1* haploinsufficiency led to tetraploidization via mitotic bypass and subsequent whole-genome endoreduplication.[4]

Chk1 is a Ser/Thr kinase essential for cell viability, possibly through its role in monitoring the proper execution of DNA replication and mitosis.[5] However, the role of Chk1 in these processes is still unclear. Hints of its role have emerged in experiments following exogenous DNA damage stimuli. Under these conditions, the upstream kinase ATR rapidly activates Chk1, which, in turn, will phosphorylate numerous downstream substrates promoting the coordination of DNA repair with cell cycle progression.[5,6]

In unperturbed cells, Chk1 can also be found associated with chromatin and phosphorylates Thr11 in histone H3, leading to its acetylation at Lys9, a mark of transcriptionally active regions.[7] Upon DNA damage, Chk1 disengages from chromatin with two main consequences: a decrease of histone H3 phosphorylation, leading to repression of transcription of key cell cycle regulators,[7] and an increase of inhibitory phosphorylations of CDC25, a positive regulator of cell cycle progression.[8] This one-two punch highlights the central role of Chk1 in regulating cell cycle progression.

Three main mechanisms have been proposed to explain tetraploidization in cancer: cell fusion, defects in mitosis and endoreduplication.[9] Endoreduplication has been attributed to persistent ATM/ATR-mediated DNA damage signaling resulting from telomere dysfunction, DNA repair defects or oncogene overexpression.[9] Bunz and colleagues now propose a distinct mechanism to achieve tetraploidization: high frequency of mitotic bypass due to decreased CHK1 gene dosage in p53-deficient cells. The authors demonstrate that this phenotype is ATR-independent and likely not due to DNA damage-induced effects. In fact, endogenous DNA damage levels, as judged by 53BP1 and phospho-H2AX nuclear foci formation, were similar in $CHK1^{+/-}$ and parental cells, showing that decreased levels of *Chk1* by itself do not lead to a substantial increase in DNA damage.[4]

So, what mediates mitotic bypass caused by *CHK1* haploinsufficiency? Because Cdk1 (also known as cdc2) has been shown to control entry to mitosis, the authors hypothesized that normal levels of Chk1 might be required for full activation of Cdk1. Indeed, in p53-deficient $CHK1^{+/-}$ cells, Cdk1 activity was diminished when compared with parental p53-deficient $CHK^{+/+}$ cells. Moreover, stable overexpression of Cdk1 in a p53-deficient $CHK1^{+/-}$ background suppressed the formation of tetraploid cells. Whether the observed effect of *CHK1* haploinsufficiency on Cdk1 activity is direct or not awaits further investigation.

In summary, the study by Bunz and colleagues shows that *CHK1* haploinsufficiency may play an important role in tumorigenesis by promoting genetic instability in cells that are *p53*-deficient. This work opens up a new front in the study of mechanisms of mitotic bypass and highlights the power of genetic analysis using somatic knockout approaches. The Bunz laboratory has made seminal contributions to the development of these methods and is particularly well positioned to make further inroads into these difficult-to-tackle problems.

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