

Cell Cycle News & Views

A phosphatase turns aggressive: The oncogenicity of Cdc14B

Comment on: Chiesa M, et al. *Cell Cycle* 2011; 10:1607–17

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Cdc14 was first identified as an essential regulator for mitotic exit in the budding yeast *Saccharomyces cerevisiae* in the 1970s.¹ Mammals have two Cdc14 homologs, Cdc14A and Cdc14B, with different subcellular localizations and seemingly different cellular functions.² Because of its nucleolar localization, Cdc14B was suggested to have a critical role in mitotic exit, as its yeast counterpart does. However, both human³ and mouse⁴ cells deficient in this phosphatase failed to show any evidence to support such a suggestion. In fact, CDC14B seems dispensable for cell cycle altogether. Apart from the suggested function in mitotic exit, CDC14B was shown to facilitate the bundling of microtubules in vitro.⁵ This microtubule bundling activity of CDC14B also seemed disposable for animal development.⁴ The only function revealed by genetic ablation of CDC14B is that it is required for proficient DNA damage repair, although much needs to be learned about how exactly this phosphatase helps DNA repair.^{3,4} It cannot be ruled out, however, that all of the suggested functions of CDC14B are true but redundant with its paralog CDC14A. In fact, CDC14A was also shown to be required for DNA repair.³

Given the requirement of CDC14B in DNA repair and, potentially, in the DNA damage checkpoint, it was suggested that this phosphatase is a tumor suppressor.⁵ Now comes a twist. In the May 15th issue of *Cell Cycle*, Chiesa et al.⁶ reported that overexpression of CDC14B could transform cells instead. In the classic transformation assay, the authors observed that CDC14B overexpression rendered in the cells the ability to form colonies in soft agar. When they were injected into SCID mice, these cells were able to form tumors, demonstrating the oncogenic activity of CDC14B. The authors also observed dramatic changes in cytoskeleton and focal adhesion in CDC14B-overexpressing NIH3T3 fibroblasts.

How does CDC14B transform the cells? To answer that question, Chiesa et al. profiled gene expression changes induced by CDC14B. It became obvious to the authors that CDC14B induced activation of Ras pathway, as more than 80% of the genes being differentially expressed following CDC14B overexpression were similarly regulated by H-RasV12. A large portion of these genes encode either extracellular components or plasma membrane-associated proteins. To determine whether

CDC14B indeed participates in Ras-induced transformation, the authors tested the susceptibility of Cdc14b-depleted NIH3T3 fibroblasts to the transformation by H-RasV12. Indeed, the depletion of Cdc14b expression significantly reduced the number of colonies formed in soft agar. Furthermore, inhibition of Mek1/2 activity by a small-molecule H1152 could reverse the morphological changes induced by Cdc14B-overexpression. Taken together, these results led the authors to believe that that CDC14B acted via Ras-MAPK cascade.

This study by Chiesa et al. revealed a novel function of the CDC14B phosphatase. However, the oncogenicity of CDC14B requires further support from other experimental approaches, such as transgenic mouse models, before we can conclude definitively that CDC14B is an oncogene.

References

1. Hartwell LH, et al. *Genetics* 1973; 74:267-86.
2. Mocchiari A, et al. *J Cell Biol* 2011; 123:2867-76.
3. Berdougo E, et al. *Cell Cycle* 2008; 7:1184-90.
4. Wei Z, et al. *Mol Cell Biol* 2011; 31:1470-7.
5. Bassermann F, et al. *Cell* 2008; 134:256-67.
6. Chiesa M, et al. *Cell Cycle* 2011; 10:1607-17.

Rad18 emerges as a critical regulator of the Fanconi anemia pathway

Comment on: Palle K, et al. *Cell Cycle* 2011; 10:1625–38

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Fanconi anemia (FA) is a genetic disorder resulting in defective repair of DNA damage, genomic abnormalities and the development of cancer. The disease can be caused by defects in at least 13 different genes that function within the FA pathway and is characterized by hypersensitivity to DNA interstrand crosslinking agents such as cisplatin and mitomycin c.¹ Such DNA lesions cause stalling of DNA replication forks, resulting in the activation of the FA pathway and the monoubiquitination of FANCD2 by the FA core complex that contains an E3 ubiquitin ligase. The monoubiquitination

of FANCD2 is required for FANCD2 association with chromatin, where it coordinates the repair of DNA damage by nucleotide excision repair, translesion synthesis and homologous recombination.¹

Recently, Vaziri and colleagues demonstrated that the FA pathway becomes activated in response to bulky DNA adducts induced by BPDE in a Rad18-dependent manner.² Rad18 is a conserved E3 ligase that monoubiquitinates the replication factor PCNA in response to stalled replication forks at UV-induced lesions. Monoubiquitination of PCNA recruits bypass

polymerases that allow replication to pass the lesion. Song et al. demonstrated that PCNA monoubiquitination by Rad18 was necessary for the activation of FANCD2 monoubiquitination in response to BPDE.²

Given that both Rad18- and FA-deficient cells are also sensitive to replication-induced double-strand breaks mediated by the Topoisomerase I inhibitor camptothecin (CPT), Palle and Vaziri questioned whether Rad18 was also playing a role in activation of the FA pathway in response to CPT.³ The authors showed that low doses of CPT induce FANCD2

ubiquitination, chromatin loading and assembly into foci. Depletion or absence of Rad18 dramatically reduced FANCD2 monoubiquitination and chromatin loading, both basally and after CPT treatment. This reduction in FANCD2 recruitment is functionally important, as depletion of FANCD2 or Rad18 resulted in hypersensitivity to CPT, persistent DNA damage and reduced recovery of DNA replication.

The authors go on to demonstrate that while Rad18's ubiquitin ligase activity is required to promote chromatin loading of FANCD2, PCNA monoubiquitination is not detected after treatment with CPT. These observations are similar to a recent study that reported that Rad18's ubiquitin ligase activity is required independently of PCNA monoubiquitination for the efficient monoubiquitination of FANCD2 in response to mitomycin c.⁴ Thus, Rad18 can promote FANCD2 ubiquitination by a PCNA-dependent mechanism in response to bulky DNA adducts² and a PCNA-independent mechanism in response to interstrand cross-links⁴ and replication-induced double-strand breaks.³

Why is Rad18 required for efficient FANCD2 monoubiquitination? It is unlikely that Rad18 is directly ubiquitinating FANCD2, as it is well established that the FA core complex is required for FANCD2 ubiquitination, and there is no evidence that FANCD2 is a RAD18 substrate.^{2,4} The FANCD2 deubiquitinase Usp1

has been demonstrated to be downregulated in response to DNA damage.^{5,6} The authors, however, found no evidence of CPT-induced or Rad18-mediated change in Usp1 levels and thus conclude that Rad18 is not regulating FANCD2 deubiquitination.³ Instead, Palle and Vaziri suggest that Rad18 may be necessary for FA core complex recruitment to and/or retention on chromatin.

In conclusion, Rad18 has a critical role in response to replication-fork stalling in the presence of various types of DNA damage by a number of mechanisms. In response to UV and bulky adducts, Rad18 monoubiquitinates PCNA, which then recruits bypass polymerases to allow DNA synthesis past the lesion. This leads to activation of the FA pathway and to chromatin loading of monoubiquitinated FANCD2.² In contrast, when a replication fork stalls due to an interstrand crosslink or a topoisomerase I cleavage complex, Rad18's ubiquitin ligase activity is required for proper activation of the FA pathway via an unknown mechanism.^{3,4} Lastly, Rad18 stimulates homologous recombination in an ubiquitin ligase-independent manner by interacting with and recruiting Rad51C to double-strand breaks.⁵

This work raises several interesting questions to be addressed. First, given that Rad18's role in the FA pathway is not dependent on PCNA, is there an unidentified Rad18 substrate that regulates FANCD2 monoubiquitination?

Possible mechanisms could include activating ubiquitination of FA core complex subunits or inhibiting the catalytic activity of the Usp1 deubiquitinase. Second, are there other E3 ubiquitin ligases involved in activation of the FA pathway? One such possibility is the CRL4^{Cdt2} complex which also monoubiquitinates PCNA⁷ and thus may recognize other Rad18 substrates. Next, is Rad18 regulating both basal and damage-induced monoubiquitination of FANCD2 levels by the same mechanism? Additionally, is Rad18 involved in FANCD2 monoubiquitination in response to replication stress induced by anticancer drugs such as hydroxyurea or inducers of re-replication such as MLN4924? Finally, if Rad18 is important for activating the Fanconi pathway in the presence of specific types of DNA damage, are there naturally occurring somatic or acquired mutations in Rad18 that give rise to diseases with phenotypes like Fanconi anemia? Furthermore, can mutations in Rad18 be found that affect the FA pathway without affecting Rad18's ability to monoubiquitinate PCNA?

References

1. Kee Y, et al. *Genes Dev* 2010; 24:1680-94.
2. Song IY, et al. *J Biol Chem* 2010; 285:31525-36.
3. Palle K, et al. *Cell Cycle* 2011; 10:1625-38.
4. Williams SA, et al. *Blood* 2011; 117:5078-87.
5. Huang TT, et al. *Nat Cell Biol* 2006; 8:339-47.
6. Cohn MA, et al. *Mol Cell* 2007; 28:786-97.
7. Terai K, et al. *Mol Cell*; 37:143-9.

Zinc, a promising mineral for misfolded p53 reactivation

Comment on: Puca R, et al. *Cell Cycle* 2011; 10:1679–89

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The tumor suppressor p53 is a transcription factor that, under stress signals, leads to the induction of multiple cell response, such as cell cycle arrest, apoptosis and differentiation. Notably, p53 is highly mutated in tumors, with over 50% of human malignancies harboring mutation in the p53 locus (www.iarc.fr/p53). The majority of these mutations are found in the DNA binding domain that cause "unfolding" of the p53 protein with a disruption of the wild-type p53 (wtp53) transcriptional capacity, compromising its tumor suppressor properties.^{1,2} Quite often these mutations cause the accumulation of the misfolded protein in the nucleus, making it an attractive target for therapeutic drugs, since, in theory, the refolding of such accumulated p53 may result in high

levels of active p53 able to promote apoptosis in cancer cells.

Zinc is an essential mineral that is integral to many proteins and transcription factors, key regulators of cellular functions, such as the response to oxidative stress, DNA replication, DNA damage repair, cell cycle progression and apoptosis. In particular, several proteins involved in DNA damage signaling and repair, replicative enzymes, such as DNA and RNA polymerases, and transcription factors, such as p53, require zinc for proper function.³ Interestingly, several studies reported that low intracellular zinc level was associated with increased p53 protein expression in different cells lines, as seen by the use of zinc chelator or by feeding cells with zinc-depleted

media.⁴ Notwithstanding, experiments suggest that the accumulated p53 may be dysfunctional, and hence, the wtp53 functions would be severely compromised; indeed, a marked reduction in the ability of p53 to bind downstream DNA targets was found along with zinc deficiency.⁵ Thus, zinc deficiency may render the p53 protein in a "mutant-like" conformation that will alter the ability of the cells to appropriately respond to stress insults (i.e., DNA damage).

Puca et al.,⁶ in the May 15th issue of *Cell Cycle*, fused elements of these strands and highlights a new potential application of zinc in the treatment of mutant p53-carrying tumors. The authors previously reported that depletion of HIPK2 (homeodomain-interacting

protein kinase 2), the p53 apoptotic activator, leads to deregulation of high zinc binding proteins, metallothioneins, with shifting of wtp53 proteins into misfolded "mutant-like" conformation. Notably, zinc supplementation in HIPK2-depleted cells reverted the wtp53 conformation.^{7,8} Here, the authors extended their study and explored whether zinc supplementation might similarly affect missense mutated p53 proteins in tumor cancer cells endogenously expressing well-characterized p53 mutations: contact DNA binding R273H (U373MG) and conformational R175H (SKBR3) mutations. The authors showed that zinc supplementation in combination with common anti-tumor drugs restores chemosensitivity in tested cells. Using molecular approaches, the authors showed that zinc induces refolding of mutant p53 (mutp53) protein, as assessed by increased reactivity to PAb1620 antibody, re-establishing wtp53 binding to its canonical target promoter (i.e., p21) with abolishment

of p53 recruitment onto mutp53 target gene promoters (i.e., MDR1) in response to drug treatment.

Since it has been well demonstrated that mutp53 proteins exert pro-oncogenic activity through complex formation with p53 family members (p63/p73), the authors showed that zinc disrupts the mutp53/p73 interaction, restoring wtp53 and likely also p73-mediated antitumor effects in response to drugs; as further confirmed with U-373MG *xenograft* tumors, a significant reduction in tumor volume was observed upon zinc and drug combination compared to drug treatment alone.

During the past few years, several efforts have been dedicated to the identification of small molecules for rescue of p53 functions. Indeed, being grossly and selectively expressed in tumor tissue, mutp53 represents an especially attractive target for tumor therapy. Drugs able to target mutp53 should exert toxic effects in a tumor but not in normal cells,

where mutp53 is absent. Accordingly, D'Orazi's group demonstrates that zinc supplementation in combination with anti-tumor drugs might represent a promising new strategy for modifying the equilibrium of p53 mutants towards wild-type conformation, restoring wtp53 apoptotic response to drugs. Overall, although more studies are required to answer to the several questions raised, as well dissected by the authors in session discussion, zinc might constitute a compelling molecule that deserves further exploration to understand its potential application for tumor therapy.

References

1. Bullock AN, et al. *Nat Rev Canc* 2001; 1:68-76.
2. Harris SL, et al. *Oncogene* 2005; 17:2899-908.
3. Dong J, et al. *Biochem J* 1999; 337:311-7.
4. Ames BN, et al. *Mut Res* 2001; 457:7-20.
5. Ho E, et al. *Proc Nat Acad Sci USA* 2002; 99:16770-5.
6. Puca R, et al. *Cell Cycle* 2011; 10:1679-89.
7. Puca R, et al. *Cancer Res* 2008; 68:3707-14.
8. Puca R, et al. *Exp Cell Res* 2009; 315:67-75.

Cdc14B: When a good kid turns bad

Comment on: Chiesa M, et al. *Cell Cycle* 2011; 10:1607-17.

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Although identified in the famous cell division cycle (CDC) screen by Lee Hartwell in 1971,¹ the protein phosphatase Cdc14 did not enter the limelight until the late 1990s, when work in *S. cerevisiae* unveiled an essential role of the phosphatase in regulating mitotic exit.² Exit from mitosis is defined as the transition between the completion of chromosome segregation and G₁, and requires the inactivation of mitotic cyclin-dependent kinase (CDK) complexes and the dephosphorylation of their substrates.

During the last decade, however, research into Cdc14 has accelerated; yeast Cdc14 was shown to be involved in a plethora of activities including anaphase spindle stabilization, nuclear positioning and the segregation and silencing of ribosomal DNA and telomeres.^{3,4} If elucidating the multiple roles of the phosphatase in yeast proved complicated, it is even more difficult to obtain a clear picture of the functions carried out by its vertebrate counterparts. Although Cdc14 belongs to a highly conserved family of phosphatases it remains unclear whether the yeast and vertebrate Cdc14 share common functions.³ Complicating matters is the observation that, in organisms

other than yeast, multiple paralogs of the phosphatase exist. For example, in mammals, two Cdc14 paralogs, CDC14A and CDC14B, have been identified.^{5,6} Surprisingly, both are dispensable for mitotic exit.³ Although other phosphatases, including calcium-dependent calcineurin, PP1 and PP2A,⁷ may reverse CDK-mediated phosphorylation in Metazoans, the final word on the contribution (or not) of Cdc14 phosphatases to mitotic exit awaits the analysis of the consequences of eliminating both CDC14A and CDC14B.

Functional analyses of Cdc14 paralogs also revealed myriad roles for the phosphatases. Depending on the study and the methodologies used (e.g., depletion by RNAi, knockout or overexpression), different and sometimes controversial outcomes have been obtained. Cdc14B, the functional ortholog of the yeast phosphatase, has been proposed to play a role in nuclear organization, mitotic spindle assembly, centriole duplication, mitotic exit, G₂ DNA damage checkpoint activation and DNA repair (reviewed in ref. 3). The involvement of Cdc14B in multiple processes is consistent with the need of nature to recycle and adapt its few phosphatases to oppose their

outnumbering kinases. As such, the discrete phenotypes observed in knockdown experiments may be misleading, as it once was for yeast Cdc14, as we now know that it mediates activities beyond mitotic exit.

To an already complex scenario Chiesa and colleagues have now added another twist to the story. In their study published in the May 15th issue of the journal *Cell Cycle*, the authors showed that transfecting Cdc14B into NIH-3T3 mouse fibroblasts led to cellular transformation.⁸ The tumorigenic potential of fibroblasts overexpressing Cdc14B was tested and confirmed in mouse xenografts and chicken embryos, as injection of Cdc14B-overexpressing cells resulted in the formation of tumor masses.⁸ This phenotype was due to Cdc14's phosphatase activity, as neither the overexpression of Cdc14A or a phosphatase-dead allele of Cdc14B caused transformation.

What is the molecular mechanism that underlies the oncogenic potential of Cdc14B? A hint came from the observation that the morphological changes associated with overexpressing Cdc14B were reminiscent of those observed in cells overexpressing the Ras onco-

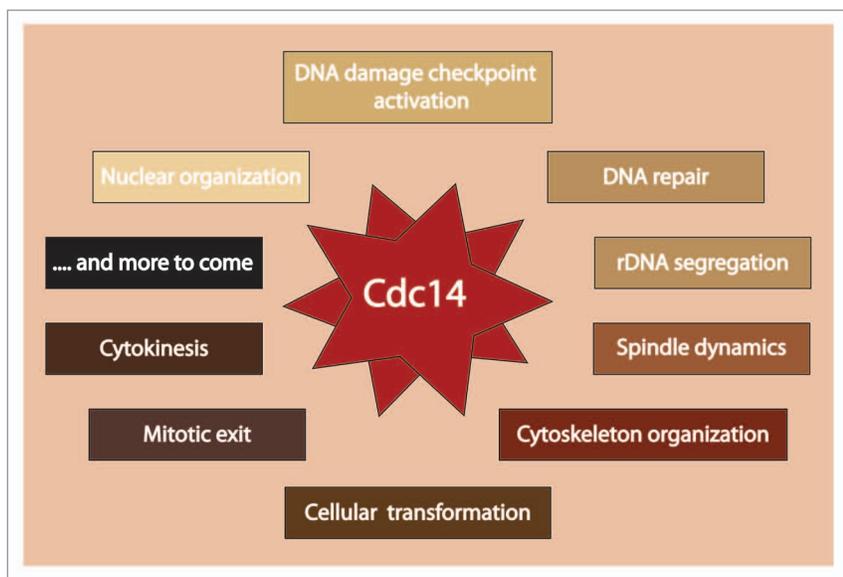


Figure 1. The multiple functions of Cdc14 phosphatases. Work from different species has linked Cdc14 to a wide variety of cellular processes portraying a somewhat confusing picture of the Cdc14 phosphatase family and its functions. Likely in the years to come additional roles will be unveiled explaining how nature has recycled and successfully adapted a single phosphatase to satisfy multiple purposes.

protein H-RasV12. These included anchorage-independent growth, loss of contact inhibition and changes in cell shape.⁸ To explore the link between Cdc14B and H-RasV12, the authors investigated and compared the cellular transcription profiles caused by overexpression of the two oncoproteins in stable NIH-3T3 clones. Similar deregulations in gene expression were found. These data combined with

the finding that co-expressing Cdc14B and H-RasV12 did not give an exacerbated phenotype led the authors to conclude that Cdc14B and H-RasV12 may act in a common pathway. Additional analyses by Chiesa et al. suggested that, among the many signaling pathways activated by Ras,⁹ the Ras-Raf-Mek pathway may be mainly responsible for mediating the oncogenic effects of Cdc14B. Although the

details of the mechanism by which Cdc14B elicits cellular transformation remain to be determined, the new findings are intriguing, as they suggest that mammalian phosphatases, besides acting as tumor suppressors (e.g., PP2A; PTEN), may also function as oncogenes (e.g., CDC14B).

Forty years after its discovery, the Cdc14 phosphatase continues to surprise. Functional clues coming from different species suggest that the phosphatase is used in various pathways aimed at fine tuning growth and cell division. Future identification of CDC14B substrates and interactors as well as the characterization of the spatio-temporal control of its activity promise to reveal which events CDC14B coordinates in higher eukaryotes. What is now seen as difficult to comprehend or even controversial may soon make sense and explain how a generally good kid can turn bad.

References

1. Culotti J, et al. *Exp Cell Res* 1971; 67:389-401.
2. Visintin R, et al. *Mol Cell* 1998; 2:709-18.
3. Mocciano A, et al. *J Cell Sci* 2010; 123:2867-76.
4. Rock JM, et al. *Curr Biol* 2009; 19:R1063-8.
5. Li L, et al. *J Biol Chem* 1997; 272:29403-6.
6. Krasinska L, et al. *Exp Cell Res* 2007; 313:1225-39.
7. De Wulf P, et al. *Curr Opin Cell Biol* 2009; 21:806-15.
8. Chiesa M, et al. *Cell Cycle* 2011; 10:1607-17.
9. Malumbres M, et al. *Nat Rev Cancer* 2003; 3:459-65.

It's all about flaps: Dna2 and checkpoint activation

Comment on: Budd ME, et al. *Cell Cycle* 2011; 10:1690-8

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DNA flaps function as intermediates in DNA metabolism. 3'-Flaps are generated as intermediates for homologous recombination. 5'-flaps, on the other hand, arise as necessary but dangerous DNA structures during DNA replication and DNA damage response. 5'-Flaps are generated either through strand displacement synthesis by a DNA polymerase or through the action of a DNA helicase, and generally, their degradation by 5'-nucleases is tightly coupled to their formation (Fig. 1A). Members of the family of 5'-flap endonucleases, as exemplified by FEN1, cut such 5'-flaps. Recently, however, another type of 5'-endonuclease, Dna2, has gained attention because of its involvement in multiple pathways involving the degradation

of 5'-flaps. In the May 15th issue of *Cell Cycle*, Budd et al. showed that deletion of *S. cerevisiae* Dna2 leads to a permanent checkpoint arrest, resulting in a lethality that can be alleviated by the inactivation of key cell cycle checkpoint factors.¹ In fact, it had been known for over a decade that the temperature sensitivity of *dna2* hypomorphs could be suppressed by deletion of the checkpoint mediator RAD9.² However, at that time, the mechanistic significance of this result was unclear, since the role of Dna2 nuclease in DNA metabolism had not yet been delineated.

Dna2 was originally identified as a DNA helicase with an associated nuclease activity, but subsequent studies showed that its

nuclease activity performs the primary function in the pathways studied and is essential for viability.^{3,4} The function of the helicase appears to be more ancillary in character. During Okazaki fragment maturation, 5'-flaps are generated through strand displacement synthesis by DNA polymerase δ .⁵ Most flaps remain small and undergo concomitant cleavage by FEN1, but some flaps escape cleavage and grow to a length of >25 nt, which allows coating by the single-stranded binding protein RPA. These RPA-coated flaps are refractory to FEN1 cutting, but they become substrates for Dna2. Since Dna2 cutting is not precise but leaves a small 5'-flap of 2-6 nucleotides, further trimming by FEN1 or another

flap endonuclease is still necessary (Fig. 1B). Similarly, during long-patch base excision repair in the mitochondria of mammalian cells, flaps generated by DNA polymerase γ are cut by FEN1 when they are small and by Dna2 when they are longer.^{6,7} Most recently, resection of the 5'-strand at a double-stranded break has been shown to be mediated by the coupled action of Sgs1 helicase and the Dna2 nuclease.^{8,9} This degradation process is unique in that FEN1 is apparently not involved. None of these pathways show a substantial dependence on the helicase function of Dna2.

Okazaki fragment maturation proceeds primarily through the generation of short flaps that are cut by FEN1, with the Dna2-dependent long flap pathway being a rare backup mechanism.¹⁰ Biochemical and genetic studies have shown that several factors can influence the balance of short versus long flaps during Okazaki fragment maturation (Fig. 1B).⁵ This balance is an important

consideration, because there is good evidence that the checkpoint-mediated lethality of a *DNA2* deletion is caused specifically by the accumulation of long flaps during Okazaki fragment maturation. Lethality of *dna2-Δ* is suppressed by mutations and conditions that limit the accumulation of long flaps on the lagging strand of the replication fork; for instance, by the reduction in efficiency with which Pol δ carries out strand displacement synthesis (in *pol32-Δ*, that deletes its third subunit), or by deletion of *PIF1*, a helicase which actually promotes the generation of longer flaps.¹¹ In addition, overexpression of *RAD27* (encoding FEN1) increases the utilization of short flaps, thereby also limiting the accumulation of long flaps and thus suppressing the lethality of *dna2-Δ* hypomorphs. In contrast, the phenotypic defects caused by an increase in the generation of long flaps, either in a *rad27-Δ* mutant or in a proofreading-defective mutant of Pol δ , which results in increased strand displacement

synthesis, can be rescued by overexpression of *DNA2*. Finally, the balance of short versus long flaps can also be affected by post-translational modification of FEN1 and Dna2.¹²

Given the high importance assigned to the FEN1 pathway, one may wonder why a *rad27-Δ* mutant is viable but *dna2-Δ* is not. Budd et al. show that the answer lays in checkpoint activation.¹ Rad27 deficiency is partially compensated by the action of another flap exonuclease, Exo1, and partially by Dna2 cutting of the longer flaps, which are generated more frequently in the *rad27-Δ* mutant. However, the *rad27-Δ* defect also leads to the generation of double-stranded breaks which require homologous recombination for repair, hence the lethality of *rad27-Δ rad52-Δ* double mutants. On the other hand, Dna2 deficiency leads to the persistent accumulation of long 5'-flaps, and these RPA-coated flaps likely serve as recruitment sites for the principal checkpoint kinase Mec1, initiating a continual checkpoint response with subsequent lethal consequences in G₂/M. Remarkably, both the DNA damage checkpoint, which proceeds via the Rad9 mediator, and the replication checkpoint, which proceeds via the Mrc1 mediator, contribute to the terminal arrest of a *DNA2* deletion, since maximal growth is recovered when the checkpoint functions of both mediators are eliminated. The latter observation is an indication that Okazaki fragment maturation is tightly coupled to its maturation by FEN1, Dna2 and DNA ligase, since failure in maturation leads to replication-fork stalling with associated activation of the Mrc1-dependent checkpoint pathway.

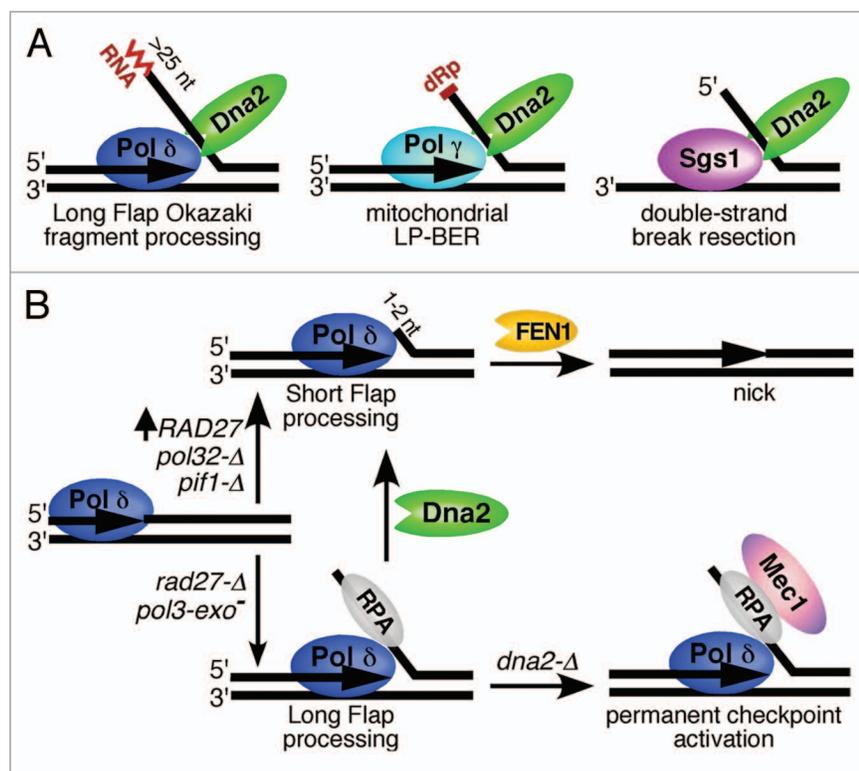


Figure 1. Dna2 nuclease in DNA metabolism. (A) Pathways in which Dna2 nuclease participates. (B) Balance of short versus long flap distribution during Okazaki fragment maturation is affected by various mutations. RAD27 = FEN1. See text for details.

References

- Budd ME, et al. Cell Cycle 2011; 10:1690-8.
- Formosa T, et al. Genetics 1999; 151:1459-70.
- Budd ME, et al. J Biol Chem 1995; 270:26766-9.
- Lee KH, et al. Nucleic Acids Res 2000; 28:2873-81.
- Burgers PM. J Biol Chem 2009; 284:4041-5.
- Zheng L, et al. Mol Cell 2008; 32:325-36.
- Duxin JP, et al. Mol Cell Biol 2009; 29:4274-82.
- Cejka P, et al. Nature 2010; 467:112-6.
- Niu H, et al. Nature 2010; 467:108-11.
- Ayyagari R, et al. J Biol Chem 2003; 278:1618-25.
- Pike JE, et al. J Biol Chem 2009; 284:25170-80.
- Balakrishnan L, et al. J Biol Chem 2009; 285: 4398-404.

GM130: New insights into oocyte asymmetric division

Comment on: Zhang CH, et al. *Cell Cycle* 2011; 10:1639–54

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Different from symmetric cell division in mitosis, mammalian oocyte meiosis is characterized by a unique asymmetric division, producing a highly polarized big MII-arrested oocyte and a small polar body. This process is critical for the retention of maternal components for early development. Asymmetric division depends on oocyte polarization, which includes peripheral spindle positioning and cortical reorganization. After GVBD, the centrally formed spindle migrates to the oocyte cortex in an actin-dependent way. Furthermore, microfilaments are enriched to form an actin cap, cortical granules (CGs) are redistributed to form a CG-free domain (CGFD), and microvilli are lost in the region overlaying the spindle. When cortical reorganization is completed, the oocyte extrudes the first polar body, leaving a highly polarized egg. Interestingly, oocyte polarization is independent of any external ligand, and the signal is intrinsic to the oocyte.

To date, the molecular details of oocyte polarization and asymmetric division are still poorly understood. Only a few molecules are identified to be involved in this process.^{1,2} Moreover, recently our group showed that Arp2/3 complex, JMY and WAVE2 are involved in spindle positioning and asymmetric division in mouse oocytes.³⁻⁵ Now, a new finding from Zhang et al.⁶ shows a novel molecule that is involved in this process. In mitosis, GM130, a part of cis-Golgi matrix, is involved in ER-Golgi transport and centrosome-related processes. In their paper, Zhang et al. found that GM130 shows a dynamic localization pattern and localizes at the meiotic spindle poles at metaphase in mouse oocytes. A functional study using MO injection indicated that GM130 is required for spindle formation, polar body extrusion and asymmetric division. Defective spindle formation was observed after GM130 depletion, indicating that GM130 is involved in microtubule dynamics. Moreover, they found that GM130

depletion causes the dispersion of p-MEK, γ -tubulin and Plk1 from spindle poles, confirming the roles of GM130 in spindle formation. However, the relationship among these molecules is still not clear, for depletion of any molecule could affect spindle organization, which would disrupt the localization of the other molecules to their proper position. It is hard to determine whether this is caused by the spindle disruption or by the interaction among molecules. Their following study confirmed the relationship between GM130 and actin: spindle migration is disrupted, the big polar body is extruded due to the failure of asymmetric division, and the actin cap is not organized in GM130-depleted oocytes, indicating the failure of oocyte polarization. These processes are all generally thought to result from actin abnormality. Since the MAPK pathway has been proved to regulate polar body size in oocytes,⁷ the authors suggest that GM130 may act as a upstream regulator of MAPK in this process. A question raised that it is still unclear is whether the spindle disruption after GM130 depletion is due to the abnormal actin dynamics.

GM130 is shown to bind Cdc42 in mitosis. Since Cdc42 is an activator of Arp2/3 complex, GM130 may be involved in the regulation of Arp2/3 complex. An activator of Arp2/3 complex, WAVE2, is regulated by MAPK,⁸ indicating that a GM130-MAPK-WAVE2-Arp2/3 complex pathway may exist. Moreover, WHAMM,⁹ a new identified activator of Arp2/3 complex, also localizes to the cis-Golgi apparatus and is involved in actin assembly. Combined with this new finding, it is reasonable to hypothesize that molecules related to the cis-Golgi apparatus may also be involved in Arp2/3 complex-mediated actin assembly, which further affects oocyte asymmetric division (Fig. 1). In summary, the finding from GM130 extends our knowledge about the oocyte asymmetric division and links the cis-Golgi apparatus to this process. The relationship

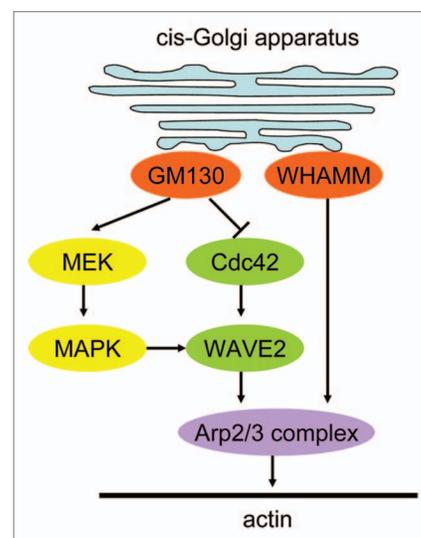


Figure 1. Diagram of GM130 pathway. GM130 binds to Cdc42 to activate WAVE2, an activator of Arp2/3 complex for actin nucleation, and, thus, a GM130-Cdc42-WAVE2-Arp2/3 complex pathway may exist; GM130 regulates localization of MEK, while MEK is an upstream regulator of MAPK, which is required for WAVE2 activity. WHAMM, which also localizes to the cis-Golgi apparatus, is an activator of Arp2/3 complex for actin nucleation. This finding provides the relationship between the cis-Golgi apparatus molecules and actin nucleation.

between cis-Golgi apparatus molecules (like GM130 and WHAMM) and actin-related molecules (like WAVE2, Arp2/3 complex) needs to be investigated in future.

References

1. Brunet S, et al. *Hum Reprod Update* 2011; 17:68-75.
2. Azoury J, et al. *Biol Cell* 2009; 101:69-76.
3. Sun SC, et al. *PLoS ONE* 2011; 6:e18392.
4. Sun SC, et al. *Mol Hum Reprod* 2011; 17:296-304.
5. Sun SC, et al. *Cell Cycle* 2011; 10:1853-60.
6. Zhang CH, et al. *Cell Cycle* 2011; 10:1639-54.
7. Choi T, et al. *Proc Natl Acad Sci USA* 1996; 93:7032-5.
8. Mendoza MC, et al. *Mol Cell* 2011; 41:661-71.
9. Campellone KG, et al. *Cell* 2008; 134:148-61.

SAYP: A bridge connecting specific and general transcription regulators

Comment on: Vorobyeva NE, et al. *Cell Cycle* 2011; 10:1821–7

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Metazoan development depends on the precise orchestration of spatially and temporally regulated gene expression programs. A well-studied paradigm of developmentally regulated gene expression is the ecdysone cascade in *Drosophila*. The hormone 20-hydroxyecdysone is produced and binds to a heterodimer consisting of the ecdysone receptor and the ultraspiracle protein in third instar larvae.¹ This results in the execution of an intricate transcription cascade that prepares and guides larvae through the following stages of development, pupariation and metamorphosis. Some ecdysone-regulated genes are expressed at the larval stage, when ecdysone levels are still relatively low. Many of these early response genes encode themselves transcription factors, for example DHR3, that activate genes during later stages of the process. DHR3, like the ecdysone receptor itself a member of the nuclear receptor superfamily, plays a central role in the switch from early to late gene activation.² It binds to and activates late genes such as *FTZ-F1* which is expressed during the mid- to late prepupal period. Premature activation of *FTZ-F1* by DHR3 is prevented by its physical interaction with yet another hormone receptor, E75B.³

While these subsequent and interdependent actions of different nuclear hormone receptors lie at the core of the ecdysone cascade, these transcription factors do not work in isolation. In order to activate or repress their target genes they need to engage with factors regulating chromatin structure and with general transcription factors that facilitate binding of RNA polymerase II. The ecdysone receptor has been shown to interact with Taiman, Dek

and NURF,^{4,5} but little is known about the mechanism of action of hormone receptors like DHR3 that act during later stages of the ecdysone cascade.

An elegant study by Vorobyeva and colleagues has now provided new insights into DHR3 function.⁶ They have demonstrated that DHR3 binds SAYP, a subunit of the BTFly complex.⁷ In addition to SAYP, this complex contains members of the PBAP chromatin remodeling and the TFIID complexes and, thus, combines chromatin remodeling and general transcription factors.⁸ The role of SAYP and BTFly in gene regulation has been well established, but how this multifunctional complex finds its way to the genes it regulates has remained enigmatic. The new study shows that DHR3 forms a high molecular weight complex with BTFly *in vivo*, suggesting that DHR3 might recruit BTFly to promoters in an ecdysone-dependent manner. In support of this hypothesis, DHR3 and SAYP localize to overlapping sets of bands on polytene chromosomes and co-occupy promoters of several genes. Interestingly, depletion of SAYP does not interfere with DHR3 binding but prevents recruitment of other BTFly subunits and RNA polymerase II. This demonstrates that SAYP serves to bridge DHR3 and BTFly.

That DHR3 and SAYP work together is underscored by the finding that both genes cooperate genetically. Decreased pigmentation of bristles in the y^2 mutant caused by loss of SAYP function is exacerbated by loss of DHR3 function. However, it is unlikely that these phenotypes are related to ecdysone signaling. Therefore, these results point towards a more general relationship between SAYP and

DHR3. Indeed, previous genomewide transcriptome analyses have suggested that both proteins regulate a large number of genes of which ecdysone regulated genes constitute a minority.^{9,10} It might be interesting to compare these data sets, in order to assess the overall contribution of DHR3 for SAYP and BTFly chromatin binding.

DHR3 is the first transcription factor shown to recruit BTFly, but it is unlikely to be the only one. Do other hormone receptors acting in the ecdysone cascade also engage BTFly, or is this a property specific for DHR3? The results also suggest a potential mechanism for the inhibition of DHR3 by E75B mentioned above. It is conceivable that SAYP and E75B interact with overlapping surfaces of DHR3, and that the mechanism of E75B repression involves displacement of BTFly from promoter-bound DHR3.

The study by Vorobyeva and her colleagues from the Shidlovskii lab have revealed an unexpected link between nuclear hormone receptors and BTFly and has raised a number of interesting questions that are likely to further our molecular understanding of both hormone induced and general gene activation processes during development.

References

1. Thomas HE, et al. *Nature* 1993; 362:471-5.
2. Huet F, et al. *Dev* 1995; 121:1195-204.
3. White KP, et al. *Science* 1997; 276:114-7.
4. Sawatsubashi S, et al. *Gen Dev* 2010; 24:159-70.
5. Badenhorst P, et al. *Gen Dev* 2005; 19:2540-5.
6. Vorobyeva NE, et al. *Cell Cycle* 2011; 10:1821-7.
7. Shidlovskii YV, et al. *EMBO J* 2005; 24:97-107.
8. Vorobyeva NE, et al. *PNAS* 2009; 106:11049-54.
9. Chalkley GE, et al. *Mol Cell Biol* 2008; 28:2920-9.
10. Ruaud AF, et al. *Dev* 2010; 137:123-31.

Breast tumor microenvironment: In the eye of the cytokine storm

Comment on: Martinez-Outschoorn UE, et al. *Cell Cycle* 2011; 10:1784–93

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There is increasing evidence that cancer progression is sustained by signals emanating from tumor microenvironment. Several cell populations such as fibroblasts, immune cells, adipocytes, or endothelial cells compose the

tumor niche. Even if the complex molecular dialogue between all the components of the tumor microenvironment is poorly understood, it is well accepted that tumor considerably modified its stroma. Cancer-associated

stroma gene expression signatures have been established for breast cancer. Interestingly, gene expression from the tumor stroma generates clusters linked to clinical outcome.¹ This suggests that changes in breast tumor stroma

play a role in disease progression and outcome. Understanding the molecular interplay between stroma cells and cancer cells should help to develop new anti-cancer therapies.

Recently, Lisanti's lab has characterized different inflammatory mediators and growth factors secreted by tumor fibroblasts (TFs).² They designed a co-culture system to monitor fibroblast activation by MCF7 cancer cells. The crosstalk between the two cell populations induces an oxidative stress in fibroblasts that triggers a massive release of cytokines through NF κ B activation. This local cytokine storm induces autophagy in TFs. Autophagic destruction of TFs produces nutrients that can be recycled by anabolic cancer cells. In addition to autophagy in TFs the cytokine release may directly activate the cancer stem cell (CSC) population. Several interleukins, including IL4, IL6 and IL8, are known to stimulate CSCs self-renewal inducing tumor progression and metastasis formation.^{3,4}

Actually, the cytokine/growth factor interplay between TFs and cancer cells may be more general and involve different cell players such as mesenchymal stem cell (MSCs) and tumor endothelial or lymphocytic cells. Developing tumors may recruit MSCs from the bone marrow where they interact with and regenerate breast CSCs. An IL6-mediated chemotaxis may

facilitate the homing of MSCs to the sites of primary tumor growth. MSC-derived CXCL7, in turn, interacts with mature cancer cells through the CXCR2 receptor, where it induces the synthesis of a number of cytokines, including IL6 and IL8.⁵ Another important consequence of this local cytokine storm is the induction of a cancer-related inflammation. The level of tumor macrophages (TMs) is frequently associated with poor prognosis.⁶ Recruitment of inflammatory cells to the tumor microenvironment seems to play an important role in tumor progression by promoting angiogenesis and inhibiting adaptive immunity. A recent study suggests that TMs directly dialogue with cancer cells via a cytokine-mediated crosstalk involving CCL18 and inducing cancer cell invasion and metastasis formation.⁷ Together, these diverse lines of evidence demonstrate that cancer cells actively dialogue with their niche via a complex cytokine network supporting tumor progression. Unfortunately, massive cytokine release may occur in breast tumors exposed to cytotoxic chemotherapy. This may contribute to the resistance of certain cancer cells to current treatments. Thus, interfering with cytokine release may be a good therapeutic strategy to treat cancer and prevent tumor relapse. The potential of anti-inflammatory therapies to treat breast cancer has been studied using an

anti-CXCR1 inhibitor.⁸ The IL8 receptor/CXCR1 blockade selectively depletes the CSC population CXCR1-positive in human breast cancer xenograft. Furthermore, this was followed by the induction of massive apoptosis in the bulk tumor population via a FAS ligand bystander effect. In addition, CXCR1 blockade was able to reduce metastasis formation. The efficiency of such therapeutic strategy may be generalized to other cancer types. For example, blockade of CXCR3 inhibits metastatic spread of colon cancer.⁹

All together, these observations suggest that the development of therapies interfering with the cytokine-mediated dialogue between the cancer cells and the tumor microenvironment may provide a novel means of targeting cancer, and that this interaction may be driven through CSC signaling.

References

1. Finak G, et al. *Nat Med* 2008; 14:518-27.
2. Martinez-Outschoorn UE, et al. *Cell Cycle* 2011; 10:1784-93.
3. Charafe-Jauffret E, et al. *Cancer Res* 2009; 69:1302-13.
4. Sansone P, et al. *J Clin Invest* 2007; 117:3988-4002.
5. Liu S, et al. *Cancer Res* 2011; 71:614-24.
6. Witkiewicz AK, et al. *Cell Cycle* 2009; 8:1654-8.
7. Chen J, et al. *Cancer Cell* 2011; 19:541-55.
8. Ginestier C, et al. *J Clin Invest* 2010; 120:485-97.
9. Cambien B, et al. *Br J Cancer* 2009; 100:1755-64.

Fuel for cancer cells?

Comment on: Whitaker-Menezes D, et al. *Cell Cycle* 2011; 10:1772–83

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In the 1920s, at a time when lactate (La⁻) was considered to be a marker of insufficient O₂, Otto Warburg described how cancer cells, unlike normal cells, produced significant amounts of La⁻ under resting conditions even in the presence of ample O₂ for mitochondrial oxidative metabolism.¹ This type of metabolism has been called aerobic glycolysis or the Warburg effect. Over the next 60+ years, numerous lines of evidence in the area of exercise physiology revealed that skeletal muscle cells displayed similar behavior under conditions of elevated activity; i.e., muscles, especially those with a glycolytic profile, produced La⁻ on a net basis even in the absence of dysoxia.^{2,3} Perhaps more surprisingly, however, muscles, particularly those with an oxidative profile, were often net consumers of La⁻ even

during increased contractile activity.⁴ On the basis of these results, Brooks introduced the cell-to-cell La⁻ shuttle in 1984.⁵ In this scenario, La⁻ is a dynamic energy metabolite that circulates throughout the body and is used as a fuel by numerous, perhaps most, tissues (including both resting and contracting skeletal muscle, heart, liver, kidney, and importantly, the brain).^{4,6} Other La⁻ shuttles (e.g., between astrocytes and neurons) have now been reported also.⁷ That La⁻ has a caloric value (3.62 kcal·g⁻¹) comparable to that of glucose (3.74 kcal·g⁻¹) has long been underappreciated. Blood La⁻ flux can even exceed glucose flux during moderate-intensity exercise.^{4,6} As fuels, La⁻ and glucose clearly have overlapping functions, but for anabolic growth requirements, glucose is arguably superior to lactate

for increasing biomass.¹

More recently, Sonveaux et al. provided evidence for a La⁻ shuttle within tumors.⁸ In this model, tumor cells (Warburg type?) that are hypoxic due to being at a greater distance from O₂-supplying capillaries have a glycolytic, mitochondria-poor profile and avidly consume glucose, producing La⁻ that diffuses to more well-oxygenated tumor cells near capillaries. These aerobic tumor cells rely primarily on this La⁻ as a fuel for oxidative metabolism. Quoting Semenza, "Was there any precedent that should have alerted us to the existence of this symbiotic relationship between aerobic and hypoxic cancer cells? Of course: the well-known recycling of lactate in exercising muscle."⁹ The lesson to be learned is that, in a busy world of specialization and reductionism,

researchers in all fields should make every attempt to look across disciplines for parallel findings, more comprehensive explanations, underlying themes for all of biology, and inspiration for further study.

Now comes the Lisanti group proposing a Reverse Warburg Effect in tumors having caveolin-1 deficient stromal cells, and once again a La^- shuttle is involved.^{10,11} Presently, they report that when MCF7 breast cancer cells are co-cultured with normal fibroblasts, expression of monocarboxylate transporter 4 (MCT4, a La^- transporter) is induced in the fibroblasts. When cultured alone, these fibroblasts fail to express MCT4. Concordantly, MCT1 is upregulated in the MCF7 breast cancer cells in this co-culture. Importantly, these results may be clinically relevant; primary human breast cancer samples displayed selective expression of MCT1 in the epithelial cancer cells versus MCT4 in the tumor stromal cells.¹¹ The K_m and V_{max} of MCT1 and MCT4, and their known distribution of expression suggest that MCT4 is suited for extrusion of La^- from La^- -producing cells while MCT1 is equipped to facilitate

uptake of La^- into net lactate consuming cells. These intriguing results are consistent with the notion of glucose consumption and La^- production (aerobic glycolysis) by cancer associated fibroblasts with subsequent La^- release to and consumption by, epithelial cancer cells.

Lundberg has proposed that cancer is “hundreds or thousands of unique molecular diseases demanding targeted and combination therapies.”¹² In contrast, it appears that these multitudinous genotypes may be expressed as a much smaller number of phenotypes. Especially in this context, the observations of Warburg,¹³ Sonveaux et al.,⁸ and the Lisanti group^{10,11} impel research into phenotypic treatments for cancer such as selective MCT inhibition and selective restriction of fuel supply depending on the Warburg or Reverse Warburg nature of specific cancers.¹⁴

While we now know that most cells of the body readily use La^- as a fuel, and PET scans have been invaluable in imaging tumors that consume large amounts of glucose; determining how a cancer cell line and its immediate environment handle glucose and La^- together

might ultimately determine how to implement powerful treatments. Lisanti’s group has taken us one step closer to understanding these complex relationships and the tremendous clinical impact that targeting them might have in the near future.

References

1. Vander Heiden MG, et al. *Science* 2009; 324: 1029-33.
2. Gladden LB. Chap 14, Exercise: Regulation and Integration of Multiple Systems 1996; New York (NY): Oxford Univ Press;614-48.
3. Connett RJ, et al. *J Appl Physiol* 1990; 68:833-42.
4. Gladden LB. *Med Sci Sports Exerc* 2008; 40:477-85.
5. Brooks GA. *Comparative Physiology and biochemistry: Current Topics and Trends, vol. A, Respiration-Metabolism-Circulation* 1985; Berlin (Germany): Springer;208-18.
6. Brooks GA. *J Appl Physiol* 2010; 108:1450-1.
7. Gladden LB. *J Physiol* 2004; 558:5-30.
8. Sonveaux P, et al. *J Clin Invest* 2008; 118:3930-42.
9. Semenza GL. *J Clin Invest* 2008; 118:3835-7.
10. Pavlides S, et al. *Cell Cycle* 2009; 8:3984-4001.
11. Whitaker-Menezes D, et al. *Cell Cycle* 2011; 10: 1772-83.
12. Lundberg GD. *The Scientist* 2011; 25:31.
13. Warburg O. *Science* 1956; 123:309-14.
14. Nijsten MWN, et al. *Med Hypotheses* 2009; 73:48 51.

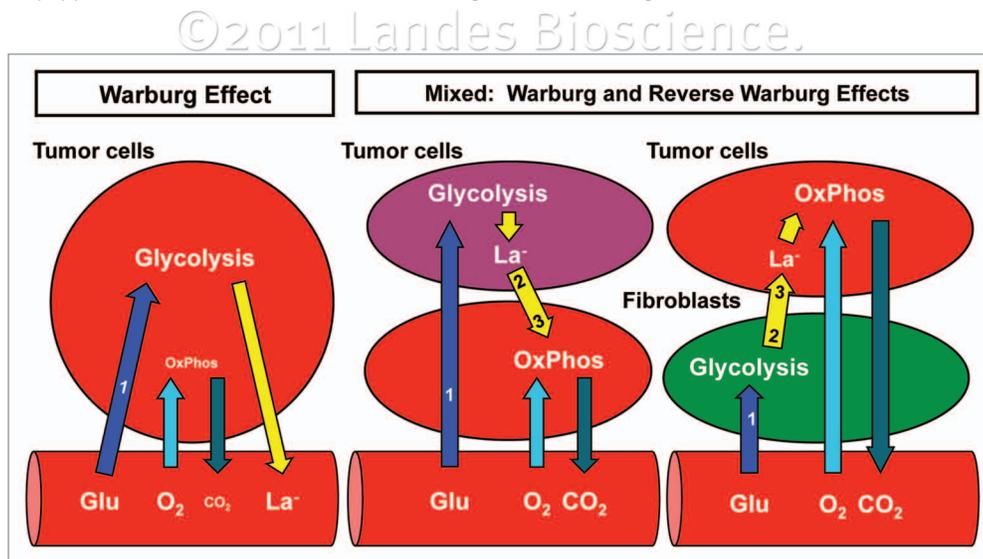


Figure 1. Three potential tumor cell phenotypes. Left panel: These are classical Warburg cancer cells.^{1,13} They avidly consume glucose (Glu) which is being supplied by capillaries and even though they are well oxygenated (O_2 , red color), they have limited capability for oxidative phosphorylation (OxPhos) and produce only small amounts of carbon dioxide (CO_2) but copious amounts of lactate (La^-). Glycolytic usage of glucose may be a superior strategy for increasing biomass. Middle panel: These are cells described by Sonveaux et al.⁸ The O_2 level decreases with distance away from the capillary (indicated by the color of the cells). The poorly oxygenated cells consume glucose and produce La^- . These poorly-oxygenated cells may be Warburg-like in that they may inadequately be equipped for OxPhos and would still be La^- -producers even if O_2 were readily available. The well-oxygenated cells (red) largely consume La^- as their fuel and may be of the reverse Warburg type in that they are not highly glycolytic and rely heavily on OxPhos for energy. Note that glucose must bypass the well-oxygenated cells for access to the hypoxic cells. Perhaps La^- out-competes glucose as a fuel in these reverse Warburg type cells? Right panel: Model of Whitaker-Menezes et al.¹¹ In this scenario, fibroblasts (green) surrounding cancer cells appear to have a Warburg-like profile, consuming glucose and producing La^- apparently with no shortage of O_2 . The cancer cells seem to be reverse Warburg-like and consume La^- as their primary fuel. Dark blue arrows labeled with “1” in all three panels indicate the potential for starving tumors by withholding glucose.¹⁴ The yellow arrows labeled with “2” in the middle and right panels indicate the possibility of inhibiting monocarboxylate transporter 4 (MCT4) to restrict La^- efflux from La^- -producing cells while the “3” in the same arrows indicates the possibility of inhibiting MCT1 to limit La^- uptake into La^- -consuming cells.^{8,11}