2-15-2012

TRPV1 antagonists may exacerbate sepsis in aged mice: Should we be nervous?

Zenaide M.N. Quezado
George Washington University

Arpad Szallasi
Monmouth Medical Center, Long Branch, NJ

Recommended Citation
Quezado, Z.M., Szallasi, A. (2012). TRPV1 antagonists may exacerbate sepsis in aged mice: Should we be nervous?. Cell Cycle, 11(4), 647-648.

Cell Cycle 11:4, 645-651; February 15, 2012; © 2012 Landes Bioscience

Cell Cycle News & Views

Micronucleophagy: A new mechanism to protect against chromosomal instability?

Comment on: Rello-Varona S, et al. Cell Cycle 2012; 11:170–6; PMID:22185757; http://dx.doi.org/10.4161/cc.11.1.18564

Patricia Boya[1] and Patrice Codogno[2]; [1]CIB; Consejo Superior de Investigaciones Cientificas (CSIC); Madrid, Spain; [2]INSERM U984; University Paris-Sud 11; Châtenay-Malabry, France; *Email: pboya@cib.csic.es and patrice.codogno@u-psud.fr; http://dx.doi.org/10.4161/cc.11.4.19278

Micronuclei are small membrane-enclosed cytoplasmic bodies containing whole or fragmented chromosomes, which are formed during anaphase in aberrant mitosis and in response to genotoxic stress. In the latter scenario, the formation of micronuclei can be used to assess the toxicity of various chemicals and drugs. A report by Rello-Varona et al. in a previous issue describes the elimination of micronuclei by autophagy.[1]

Macroautophagy (hereafter referred to as autophagy) is a highly regulated cellular mechanism for degradation and recycling of cytoplasmic contents. This process begins with the formation of an autophagosome, a double membrane structure that engulfs parts of the cytosol and whole organelles, finally fusing with a lysosome to allow the degradation of the enclosed material. The final products, including amino acids, lipids and nucleotides, are released into the cytosol via permeases present at the lysosomal membrane and can then be used for anabolic reactions to maintain cellular functions. Autophagy is conserved from yeast to human and is regulated by the Atg family of proteins.[2] Autophagy is a general response to cellular stress, which mediates the clearance of dangerous cell components, such as damaged mitochondria, and intracellular pathogens, such as viral particles and bacteria.[3]

The study by Rello-Varona and colleagues demonstrates that micronuclei generated after cell cycle perturbations are surrounded by LC3- and p62-positive staining (**Fig. 1**). This phenomenon appears to be dependent on the autophagy regulators Atg7 and Atg5, as it is not observed in cells treated with siRNAs for Atg5 and Atg7. Importantly, these micronuclei contain a reduced quantity of DNA, as determined by DNA binding dyes and fluorescent histone tagging, and exhibit a discontinuous membrane, suggesting that both the nuclear membrane and DNA are degraded by autophagy. As expected, these structures were also positive for the lysosomal marker Lamp2. Interestingly, only micronuclei presenting symptoms of DNA damage were targeted, as indicated by labeling with phospho-γH2AX, a marker of DNA damage.[1]

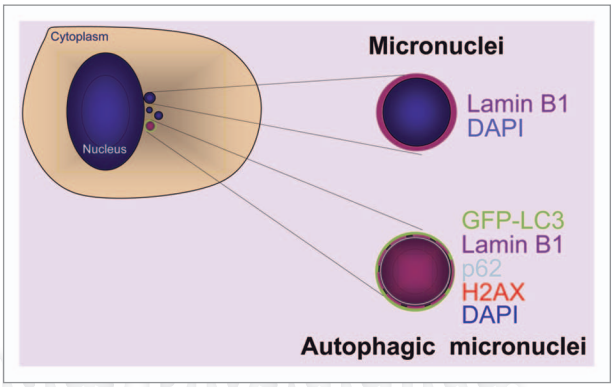

Figure 1. Schematic representation of markers during micronucleophagy.

Selective autophagy of organelles (e.g., mitochondria) and intracellular pathogens requires the recognition of LC3-interacting regions in target proteins at the surface of organelles and intracellular bacteria.[4] Interestingly, Rello et al. report that micronuclei were also positive for p62, an LC3-interacting protein,[5] whereas no p62 staining was detected in LC3-negative micronuclei. It remains to be determined whether p62 represents the bona fide receptor for this new form of selective autophagy or micronucleophagy. Moreover, further studies are required to determine how DNA damage promotes LC3 recognition, leading to the elimination of potentially harmful cellular structures.

Degradation of nuclear-derived material and even entire nuclei by autophagy has been previously described in non-vertebrates. In fact, several studies have shown that part of the nucleus can be removed by piecemeal microautophagy of the nucleus,[6] so called because no vesicular membrane-bound intermediates are created. Furthermore, in syncytial fungi the entire nucleus can be degraded by autophagy. It thus seems plausible that the size of the material undergoing degradation does not limit the nuclear degradation process. Indeed, giant LC3-positive structures are observed when *S. aureus* invades mammalian cells.[7] Selectivity, rather than the target size, thus appears to be the most important aspect of this process.

Autophagy plays an important role in limiting DNA damage and genomic instability;[8] the relevance of the findings of Rello-Varona to these processes remains to be determined. Further investigation of these phenomena, both in tumors and in autophagy-deficient cells in vivo, is thus required to determine whether micronucleophagy may be used to limit the consequences of chromosomal instability. This new form of selective autophagy adds yet more intracellular structures and organelles to the growing list of cellular components that are specifically targeted for lysosomal degradation.

References

1. Rello-Varona S, et al. Cell Cycle 2012; 11:170-6; PMID:22185757; http://dx.doi.org/10.4161/cc.11.1.18564.
2. Yang Z, et al. Curr Opin Cell Biol 2010; 22:124-31; PMID:20034776; http://dx.doi.org/10.1016/j.ceb.2009.11.014.
3. Kroemer G, et al. Mol Cell 2010; 40:280-93; PMID:20965422; http://dx.doi.org/10.1016/j.molcel.2010.09.023.
4. Dikic I, et al. Cancer Res 2010; 70:3431-4; PMID:20424122; http://dx.doi.org/10.1158/0008-5472.CAN-09-4027.
5. Pankiv S, et al. J Biol Chem 2007; 282:24131-45; PMID:17580304; http://dx.doi.org/10.1074/jbc.M702824200.
6. Krick R, et al. Mol Biol Cell 2008; 19:4492-505; PMID:18701704; http://dx.doi.org/10.1091/mbc.E08-04-0363.
7. Nakagawa I, et al. Science 2004; 306:1037-40; PMID:15528445; http://dx.doi.org/10.1126/science.1103966.
8. White E, et al. Curr Opin Cell Biol 2010; 22:212-7; PMID:20056400; http://dx.doi.org/10.1016/j.ceb.2009.12.008.

All p65-containing dimers are not equal

Comment on: Mora E, et al. Cell Cycle 2012; 11:159–69; PMID:22189654; http://dx.doi.org/10.4161/cc.11.1.18559

Simona Saccani; Max Planck Institute for Immunobiology and Epigenetics; Freiburg, Germany; Email: saccani@immunbio.mpg.de;
http://dx.doi.org/10.4161/cc.11.4.19279

Animals have evolved a strategic set of responses to control and react against infection by pathogens and tissue injury, which, together, comprise the process of inflammation.[1] Inflammation is initially a beneficial reaction for the host; however, if prolonged, becoming chronic inflammation, it can be detrimental and lead to the development or aggravation of several pathologies, including cancer.[1,2] These harmful effects of the inflammatory response have attracted considerable attention in therapeutic drug generation.

As a result, much effort has been directed at blocking the activity of one of the central players in inflammation: NFκB.[1-3] NFκB is an evolutionarily-conserved family of transcription factors that plays a crucial role in innate immunity by driving the expression of a variety of inflammatory mediators, such as cytokines and chemokines, and genes involved in pro-survival and anti-apoptotic signaling.[3]

A prolonged or persistent activation of NFκB can be harmful for an organism, yet it mediates multiple functions that are vital for health and host defense.[1-3] As a corollary, a complete block of NFκB activity would not be always advantageous. An attempt to solve this issue has been to identify drugs which selectively act only on specific downstream NFκB targets.[2]

Most NFκB targets are activated in a cell-type and stimulus-specific fashion.[3] NFκBs act in the form of homo- and heterodimers and, in some cases, target genes are activated only by particular dimer combinations, whereas others are activated redundantly by multiple different dimers.[3,4] Each NFκB dimer binds to a 9–10bp DNA motif (the "kb site"), which represents the first level of target specificity.[3,4] However, although in vitro most dimers bind to all kb sites with high affinity, it is increasingly evident that in vivo, the various dimers display different abilities to bind to particular kb sites: the rules which constrain this are under active investigation but are still not completely clear. Subsequent transcriptional activation of target genes by NFκB is also the result of multiple levels of integrated regulation, including nucleosome positioning at the target site, the effects of permissive/repressive histone modifications and post-translational modifications to NFκB itself.[3-6] In particular, it has been shown that phosphorylation of the NFκB subunit p65 at different residues has a series of transcriptional effects.[4,5,7] In keeping with current efforts to characterize drugs capable of targeting only certain aspects of NFκB activity, Mora et al.[8] showed, in a previous issue of *Cell Cycle*, that Bindarit, a proven anti-inflammatory drug, targets a specific pool of p65-containing dimers in activated macrophages. Bindarit has been in use for some years and is known to interfere with monocyte recruitment during early inflammatory responses by regulating production of the chemokine MCP1.[9] They have now shown that Bindarit causes a partial block in IκBα phosphorylation, p65 nuclear entry and its phosphorylation at S536. This results in a decrease in the transcriptional activation of MCP1[8] together with a handful of other inflammatory genes.

Importantly, many other NFκB target genes are unaffected by treatment with Bindarit. This highlights the importance of targeting specific facets of NFκB and also reveals that the decrease (but not elimination) of nuclear p65 and, in particular, of the S536-phosphorylated form, differentially affects its binding to particular kb sites at target genes. Thus, Bindarit seems to regulate inflammation by selectively modulating the activation of a subset of chemokines and, in so doing, reducing amplification of inflammation without eliminating it.[8]

While this work provides a first, fascinating glimpse of how Bindarit controls the activation of MCP1 (along with several other target genes), it raises some new, unanswered questions. Additional studies will be needed in order to identify the effector molecule(s) responsible for the reduced activation of p65-containing dimers (one possibility is that Bindarit may be acting as an inhibitor of IKKβ, which is known to phosphorylate both IkBα and p65[5]) and the extent to which Bindarit affects the activity of other NFκB dimers.

In the future, it is likely that increasing attention will be focused on developing drugs which, like Bindarit, control specific subsets of NFκB targets without disrupting the entire inflammatory system. Other areas which may be interrogated as possible drug targets include the various other post-translational modifications of the NFκB subunits as well as aspects of chromatin structure that may discriminate specific subsets of target genes.[1,2,6,8]

References

1. Karin M, et al. Nat Rev Immunol 2005; 5:749-59; PMID:16175180; http://dx.doi.org/10.1038/nri1703.
2. Grivennikov SI, et al. Cell 2010; 140:883-99; PMID:20303878; http://dx.doi.org/10.1016/j.cell.2010.01.025.
3. Hayden MS, et al. Nat Cell Biol 2004; 6:87-9; PMID:14755267; http://dx.doi.org/10.1038/ncb0204-87.
4. Natoli G. Cell 2004; 118:406-8; PMID:15315753; http://dx.doi.org/10.1016/j.cell.2004.08.003.
5. O'Shea JM, et al. Biochem Soc Trans 2008; 36:603-8; PMID:18631125; http://dx.doi.org/10.1042/BST0360603.
6. Smale ST. Cell 2010; 140:833-44; PMID:20303874; http://dx.doi.org/10.1016/j.cell.2010.01.037.
7. O'Shea JM, et al. Biochem J 2010; 426:345-54; PMID:20001970; http://dx.doi.org/10.1042/BJ20091630.
8. Mora E, et al. Cell Cycle 2012; 11:11-2; PMID:22157090; http://dx.doi.org/10.4161/cc.11.1.18559.
9. Guglielmotti A, et al. Inflamm Res 2002; 51:252-8; PMID:12056513; http://dx.doi.org/10.1007/PL00000301.

TRPV1 antagonists may exacerbate sepsis in aged mice: Should we be nervous?

Comment on: Wanner SP, et al. Cell Cycle 2012; 11:343–9; PMID:22214765; http://dx.doi.org/10.4161/cc.11.2.18772

Zenaide M. Quezado[1,*] and Arpad Szallasi[2]; [1]Children's National Medical Center; George Washington University; Washington, DC USA; [2]Monmouth Medical Center; Long Branch, NJ USA; Email: zquezado@childrensnational.org; http://dx.doi.org/10.4161/cc.11.4.19168

Desensitization of nociceptive neurons to capsaicin has a clear analgesic potential. Indeed, a high concentration capsaicin patch is already in clinical use to relieve neuropathic pain. The cloning of the capsaicin receptor TRPV1 has spurred considerable efforts in the pharmaceutical industry to find potent, small-molecule TRPV1 antagonists.[1] However, adverse effects have so far prevented any TRPV1 antagonists from advancing beyond phase II trials. In particular, concerns have surfaced around the effects of antagonizing TRPV1 on thermoregulation (hyperthermia) and on the ability to detect noxious heat (risk for scalding injury).[1] In a previous issue, Romanovsky and coworkers raised the possibility that TRPV1 blockade might also affect the response to sepsis, especially in older hosts.[2]

This is concerning, because both systemic inflammatory response syndrome (SIRS), which can occur following tissue damage, and sepsis, which occurs with microbial infection, are major public health problems and cause thousands of deaths every year.[3,4] In addition, despite extensive research into the inflammatory cascade triggered during SIRS and sepsis, the field has witnessed many failed clinical trials with drugs that alter the inflammatory response.[3] That some anti-inflammatory drugs lacked beneficial effects while others were harmful attests to the complexity and to the potential hazard of perturbing the inflammatory response.

Researchers have shown that the inflammatory response is modulated by a number of humoral and neural processes. Among the neural processes, the cholinergic anti-inflammatory pathway is long recognized.[5] More recently, TRPV1-expressing sensory neurons have emerged as potential players in modulating the inflammatory response during SIRS and sepsis. But how can TRPV1-expressing neurons play a role in inflammation? These neurons are known to release neuropeptides (e.g., substance P and calcitonin gene-related peptide) that initiate the cascade of neurogenic inflammation.[1] Indeed, TRPV1 blockade decreases neurogenic inflammation.[6]

Recent studies demonstrate that during LPS-induced SIRS in mice, TRPV1 deficiency is associated with increased inflammatory mediators and exacerbated organ damage.[7] Moreover, pharmacological TRPV1 blockade decreases survival.[8] However, the effect of TRPV1 actually varies depending on the insult (sepsis or LPS) and the mode of receptor blockade (desensitization, antagonism or gene disruption). In mice, both genetic deletion of TRPV1 and its desensitization to the ultrapotent agonist resiniferatoxin worsen survival and decrease bacterial clearance during polymicrobial sepsis but were without significant effect when LPS (without infection) triggered the inflammatory response.[8]

Studies with the relatively non-selective TRPV1 antagonist, capsazepine, yielded conflicting results. In mice with LPS-induced SIRS, capsazepine worsened survival.[8] By contrast, when administered before the onset of infection and sepsis, capsazepine actually improved survival.[9] Clearly, the role of TRPV1-expressing sensory neurons in SIRS and sepsis is incompletely understood, and the net effect of TRPV1 disruption seems to vary depending on the insult and the mode of disruption.

An underrecognized factor in TRPV1 actions is aging. For example, compared with their wild-type littermates, *Trpv1*-knockout mice are leaner when they are young but are more obese when they are getting old.[1]

Using a potent and selective TRPV1 antagonist, AMG517, Romanovsky suggests that aging may also alter the role of TRPV1 in LPS-induced SIRS.[2] While some might question the statistical power of some experiments, they confirm previous findings with capsazepine in young animals that TRPV1 antagonism worsens survival after LPS challenge. Surprisingly, this effect is reversed in older mice where AMG517 improves survival. These findings suggest that during LPS-induced SIRS, the role of TRPV1 might reverse with aging from anti-inflammatory to pro-inflammatory. Conversely, in the setting of infection, older TRPV1-deficient animals die earlier than controls, similar to findings previously reported in younger septic mice.[8]

In conclusion, aging seems to reverse the role of TRPV1 from anti-inflammatory to pro-inflammatory during SIRS but not sepsis. This is supported by the decreased serum levels of tumor necrosis factor, a known pro-inflammatory mediator, in LPS-challenged *Trpv1*-knockout older mice.[2] While it is tempting to categorize TRPV1 as anti-inflammatory vs. pro-inflammatory, one might argue that the inflammatory response in SIRS and sepsis is complex, and the presented data are far from being conclusive. Despite these issues, this is an important contribution to our understanding

of the role of TRPV1 in inflammatory response. Indeed, since TRPV1 blockade has been used clinically,[1] there is a real need to investigate the mechanisms involved more deeply. Should we be nervous? At a minimum, we should be vigilant.

References

1. Moran MM, et al. Nat Rev Drug Discov 2011; 10:601-20; PMID:21804597; http://dx.doi.org/10.1038/nrd3456.
2. Wanner SP, et al. Cell Cycle 2012; 11: In press; PMID:22214765.
3. Hotchkiss RS, et al. N Engl J Med 2003; 348:138-50; PMID:12519925; http://dx.doi.org/10.1056/NEJMra021333.
4. Martin GS, et al. N Engl J Med 2003; 348:1546-54; PMID:12700374; http://dx.doi.org/10.1056/NEJMoa022139.
5. Rosas-Ballina M, et al. Neuron 2009; 64:28-32; PMID:19840545; http://dx.doi.org/10.1016/j.neuron.2009.09.039.
6. Karai L, et al. J Clin Invest 2004; 113:1344-52; PMID:15124026.
7. Clark N, et al. FASEB J 2007; 21:3747-55; PMID:17601984; http://dx.doi.org/10.1096/fj.06-7460com.
8. Guptill V, et al. Anesthesiology 2011; 114:1190-9; PMID:21383614; http://dx.doi.org/10.1097/ALN.0b013e318212515b.
9. Ang SF, et al. Crit Care Med 2010; 38:619-28; PMID:19851090; http://dx.doi.org/10.1097/CCM.0b013e3181c0df00.

TRIM8 and p53: Making the right decision

Comment on: Caratozzolo MF, et al. Cell Cycle 2012; 11:511–23; PMID: 22262183; http://dx.doi.org/10.4161/cc.11.3.19008

Elisabetta Botti, Giulia Spallone and Antonio Costanzo*; University of Rome "Tor Vergata"; Rome, Italy; *Email: antonio.costanzo@uniroma2.it;

http://dx.doi.org/10.4161/cc.11.4.19359

Despite the growing number of functions assigned, ranging from pigmentation to fertility, the main role of the tumor suppressor protein p53 remains to preserve genome integrity by controlling two key biological outcomes of genome perturbation: the induction of cell cycle arrest, allowing DNA repair or, when the damage is irreparable, the induction of programmed cell death.[1] The choice between cell cycle arrest and apoptosis is influenced by different p53-dependent transcriptional programs that either involve cyclin-dependent kinase inhibitors (such as p21/WAF1) or apoptotic genes, such as p53AIP1, NOXA and Bax.[2]

How the decision is made at the molecular level is still the focus of intense research efforts, but it is clear that p53 post-translational modifications are key determinants of this decision: p53 phosphorylation on Ser-15 and Ser-20 residues is associated with cell cycle arrest, while Ser-46 phosphorylation is linked to cell death.[3] Protein acetylation at defined residues was also associated to the activation of apoptotic genes by p53. More recently, p53 ubiquitination, was found to affect, under certain conditions, the activity of p53 rather than stability. Ubiquitinated p53 was found in complexes bound to cell cycle arrest but not apoptosis genes, suggesting that p53 ubiquitination contributes to the selection of its transcriptional targets.[4] p53 ubiquitination is influenced by a plethora of ubiquitin ligases, most of them characterized for their ability to flag p53 for proteasome-mediated degradation.[5]

Among these, recent studies have indicated that some members of the tripartite motif (TRIM) proteins (one of the subfamilies of the RING type E3 ubiquitin ligases), which function as important regulators for carcinogenesis, are downregulated in tumors and act as important p53 regulators.[6] The RING domain of TRIM24 functions as an E3-ubiquitin ligase that targets p53 for degradation, and its depletion induces p53-dependent apoptosis.[7] The promyelocytic leukemia protein PML/TRIM19 is a p53 target that facilitates p53-Thr18 phosphorylation in response to DNA damage by recruiting p53 into PML nuclear bodies, thereby leading to p53 activation by protecting it from MDM2 inhibition. More recently, the ataxia telangiectasia group D-complementing ATDC/TRIM29 protein has been shown to bind and antagonize p53-mediated functions.[6]

In a very interesting article appeared in a previous issue of *Cell Cycle*, Caratozzolo and colleagues[8] identified in the E3 ubiquitin ligase TRIM8 as a key regulator of p53 in the cell cycle arrest vs. apoptosis decision. They showed that p53 directly activates TRIM8 transcription after DNA damage through a p53-responsive element in the first intron of TRIM8 gene. Once upregulated by p53, TRIM8 directly interacts with p53, inducing its stabilization by inhibiting MDM2 binding and, most interestingly, activating the cell cycle arrest transcriptional program but not apoptosis. This is accompanied by an increase of Ser-15 and Ser-20 phosphorylated p53 level but not of Ser-46, and, indeed, selective TRIM8 depletion facilitates DNA damage-induced apoptosis. Exogenous TRIM8 expression induced cell cycle arrest only in cell lines harboring wild-type p53 and had no effect in p53-null cells, indicating that TRIM8-induced cell cycle arrest is p53-dependent.

These findings highlight the importance of a novel feedback loop regulating the p53-dependent transcriptional program activated by DNA damage. Naturally, the findings of Caratozzolo et al. also generate questions. How does TRIM8 affect p53 ubiquitination? How are TRIM8 binding and p53 phosphorylation interconnected? Which comes first? Does TRIM8 also affect p53 acetylation? Are the other p53 family members involved in this feedback loop?

Future studies will certainly help address some of these questions and enhance our understanding of p53-related network, the ultimate beneficiaries being cancer-afflicted patients and their families.

References

1. Efeyan A, et al. Cell Cycle 2007; 6:1006-10; PMID:17457049; http://dx.doi.org/10.4161/cc.6.9.4211.
2. Das S, et al. Cell Cycle 2008; 7:154-7; PMID:18212532; http://dx.doi.org/10.4161/cc.7.2.5236.
3. MacLaine NJ, et al. Cell Cycle 2011; 10:916-21; PMID:21350334; http://dx.doi.org/10.4161/cc.10.6.15076.
4. Le Cam L, et al. Cell 2006; 127:775-88; PMID:17110336; http://dx.doi.org/10.1016/j.cell.2006.09.031.
5. Brooks CL, et al. FEBS Lett 2011; 585:2803-9; PMID:21624367; http://dx.doi.org/10.1016/j.febslet.2011.05.022.
6. Hatakeyama S. Nat Rev Cancer 2011; 11:792-804; PMID:21979307; http://dx.doi.org/10.1038/nrc3139.
7. Jain AK, et al. Cell Cycle 2009; 8:3668-74; PMID:19844164; http://dx.doi.org/10.4161/cc.8.22.9979.
8. Caratozzolo MF, et al. Cell Cycle 2012; 11:511–23; PMID: 22262183; http://dx.doi.org/10.4161/cc.11.3.19008.

Sgo1 as a "guardian spirit" for preventing colon tumorigenesis

Comment on: Yamada HY, et al. Cell Cycle 2012; 11:479–88; PMID:22262168; http://dx.doi.org/10.4161/cc.11.3.18994

Robin M. Ricke[1] and Jan M. van Deursen[1,2,*]; [1]Department of Pediatric and Adolescent Medicine; Mayo Clinic; Rochester, MN USA; [2]Department of Biochemistry and Molecular Biology; Mayo Clinic; Rochester, MN USA;*Email: vandeursen.jan@mayo.edu; http://dx.doi.org/10.4161/cc.11.4.19360

High-fidelity chromosome segregation during both mitosis and meiosis is essential for the propagation and inheritance of stable genomes. Defects in these fundamental processes promote aberrant chromosome segregation, which, in the absence of cell death, produces aneuploid progeny. In somatic cells, aneuploidy is a putative cancer-promoting event. In germ cells, aneuploidy can reduce reproductive fertility and promote the accumulation of trisomies, such as those associated with Down, Patau or Edward syndromes. In order for maintenance of genomic integrity, cells have an elaborate network of proteins that function during mitosis and meiosis to ensure accurate chromosome segregation. One such proposed mitotic regulator is shugoshin. Whereas budding yeast and Drosophila contain a single shugoshin gene, fission yeast and mammals have two paralogs (Sgo1 and Sgo2). The exact role of the shugoshin family of proteins during mitosis and meiosis has been somewhat elusive. Several functions have been proposed for Sgo1, including protecting centromeric cohesion through associating with PP2A phosphatase,[1] ensuring attachment error correction through chromosome passenger complex positioning,[2] maintaining centriole cohesion[3] and mediating kinetochore microtubule attachment by interacting directly with spindle microtubules.[4] Like Sgo1, Sgo2 has also been similarly implicated in centromeric cohesion and attachment error correction, although under different cellular circumstances than Sgo1. On the other hand, Sgo2, but not Sgo1, is thought to function during mitosis through binding spindle microtubules through its association with astrin[5] and through binding Mad2.[6] Until now, the physiological consequences of deregulated Sgo1 had been unknown.

In a previous issue of *Cell Cycle*, Yamada and colleagues set out to assess the cellular and physiological consequences of reduced Sgo1 expression,[7] since mouse Sgo1 encodes an essential gene. Importantly, which functions of Sgo1 are required for cell viability and whether this occurs at centromeres or centrosomes remains unknown. One hint that centromeric, mitotic Sgo1 may not be required for viability, however, comes from the observation that interphase Sgo1 is sufficient for the establishment of centromeric cohesion.[8] This raises the question of what the function of mitotic Sgo1 is, in addition to whether and how it contributes to chromosome segregation. Consistent with the reported roles of Sgo1, mouse embryonic fibroblasts haploinsufficient for Sgo1 harbored both amplified centrosomes as well as chromosomes that were improperly attached to spindle microtubules. Whether the attachment defect was due to aberrant geometries from centrosome amplification,[9] reduced correction of defective kinetochore microtubule interactions or precocious separation of sister chromatids is not known.

Because diminished Sgo1 expression had been previously linked to human colon neoplastic lesions, Yamada and colleagues challenged Sgo1 heterozygous mice with AOM, a carcinogen that generates DNA damage to initiate colon carcinogenesis. Importantly, mice heterozygous for Sgo1 harbored 5-fold more colon adenomas than wild-type mice at 12 weeks after completion of AOM treatment. Rather intriguingly, mice haploinsufficient for Sgo1 were actually more prone to cell death in the colonic mucosa compared with wild-type mice, at least in the initial phase of the experiment. This is a first demonstration of enhanced cell death following carcinogen challenge in a chromosomally unstable murine model. However, it remains an open question how enhanced cell death, although only immediately following carcinogen challenge, might alter the delicate balance regulating cell proliferation vs. cell death to influence tumor progression. This relationship could have significant implications in tumor etiology, progression and aggressiveness.

In summary, this study provides a causal link between diminished Sgo1 expression and induction of carcinogen-induced colon tumorigenesis. Additionally, these experiments raise a number of intriguing questions concerning the molecular mechanism for how Sgo1 contributes to high-fidelity chromosome segregation. The study of Sgo1 in mammals continues the line of investigation that began with the study of a single shugoshin gene in budding yeast and will likely lead to an increased understanding of the multiple layers of regulation necessary for proper chromosome segregation.

References

1. Xu Z, et al. J Cell Biol 2009; 187:637-53; PMID:19951914; http://dx.doi.org/10.1083/jcb.200906053.
2. Kawashima SA, et al. Genes Dev 2007; 21:420-35; PMID:17322402; http://dx.doi.org/10.1101/gad.1497307.
3. Schöckel L, et al. Nat Cell Biol 2011; 13:966-72; PMID:21743463; http://dx.doi.org/10.1038/ncb2280.
4. Salic A, et al. Cell 2004; 118:567-78; PMID:15339662; http://dx.doi.org/10.1016/j.cell.2004.08.016.
5. Dunsch AK, et al. J Cell Biol 2011; 192:959-68; PMID:21402792; http://dx.doi.org/10.1083/jcb.201008023.
6. Orth M, et al. EMBO J 2011; 30:2868-80; PMID:21666598; http://dx.doi.org/10.1038/emboj.2011.187.
7. Yamada HY, et al. Cell Cycle 2012; 11; PMID:22262168.
8. Perera D, et al. J Cell Sci 2010; 123:653-9; PMID:20124418; http://dx.doi.org/10.1242/jcs.059501.
9. Ganem NJ, et al. Nature 2009; 460:278-82; PMID:19506557; http://dx.doi.org/10.1038/nature08136

Sgo1 as a guardian of chromosome stability

Comment on: Yamada HY, et al. Cell Cycle 2012; 11:479–88; PMID:22262168; http://dx.doi.org/10.4161/cc.11.3.18994

Laura A. Diaz-Martinez and Hongtao Yu; Howard Hughes Medical Institute; University of Texas Southwestern Medical Center; Dallas, TX USA; Email: Laura.Diaz-Martinez@utsouthwestern.edu; Hongtao.Yu@utsouthwestern.edu; http://dx.doi.org/10.4161/cc.11.4.19361

Centrosome amplification and aneuploidy are commonly observed features of cancer cells. Whether these defects are a cause or a consequence of cancer development has been a subject of intense recent research. The generation of multiple knockout mouse models for genes involved in the spindle checkpoint, kinetochore-microtubule attachment or mitotic exit has provided strong evidence linking mitotic defects to chromosome instability and tumorigenesis.[1] Loss-of-function mutations of these mitotic regulators are, however, rarely found in tumor samples from human patients, suggesting that the chromosome instability observed in most tumors is due to defects in other processes. Recently, genomic studies using patient samples have implicated genes involved in chromosome cohesion as a likely source of chromosome instability in tumors.[2,3]

Chromosome cohesion is established during DNA replication in S phase and is required to keep the two sister chromatids together until anaphase, when the sister chromatids are segregated into the two daughter cells. Central to the cohesion process are the cohesin complex (which is composed of Smc1, Smc3, Scc1, and SA1/2) and multiple cohesin regulators that load (Scc2-Scc4), stabilize (Esco1/2 and sororin), protect (Sgo1) or remove (Wapl and separase) cohesin from chromosomes during the proper stages of the cell cycle. Most cohesin on chromosome arms is removed during early mitosis by mitotic kinases and Wapl. A small of pool of cohesin at the centromeres is protected by the complex between shugoshin (Sgo1) and PP2A.[4,5] This centromeric pool of cohesin is cleaved by separase following the biorientation of all sister chromatids and the silencing of the spindle checkpoint. Inactivation of Sgo1 leads to premature sister-chromatid separation in cultured human cells.[4,5]

Mutations of multiple components of the cohesion pathway, including Smc1, Smc3 and SA2 were found in human cancers.[2,3] However, whether mutations of cohesin and its regulators directly contributed to chromosome instability and tumorigenesis remained to be tested. The work by Yamada and coworkers in a previous issue of *Cell Cycle* shed light on this matter.[6] By generating Sgo- knockout mice, the authors were able to test whether reduction in Sgo1 levels resulted in chromosome instability and tumor formation. As expected, due to the key role of Sgo1 in cohesion protection, homozygous Sgo1-knockout mice were embryonic lethal. However, heterozygous Sgo1$^{+/-}$ mice were viable, and cells from these mice showed an increase in chromosome segregation defects, including chromosome misalignment, lagging chromosomes and the formation of anaphase bridges. Ultimately, these defects led to an increase in the number of aneuploid and polyploid cells, indicating that Sgo1 mutations were sufficient to cause chromosome instability. Most importantly, Sgo1$^{+/-}$ mice showed a 5-fold increase in the formation of colon tumors when challenged with a carcinogen, establishing a direct link between Sgo1 haploinsufficiency and increased tumorigenic potential.

Another phenotype observed by Yamada and colleagues in Sgo1$^{+/-}$ cells was centrosome amplification. Prior studies had implicated a short splice variant of Sgo1 (sSgo1), cohesin, and separase in regulating the centrosome cycle.[7-9] Thus, different splicing variants of Sgo1 appear to have specific roles at the centromeres and the centrosomes. Selective complementation of Sgo1$^{+/-}$ cells that are deficient for both splicing isoforms will determine the specific cellular functions of each isoform. More importantly, complementation of Sgo1$^{+/-}$ mice with different Sgo1 isoforms will reveal the individual contributions of cohesion defects and centrosome amplification to cancer development.

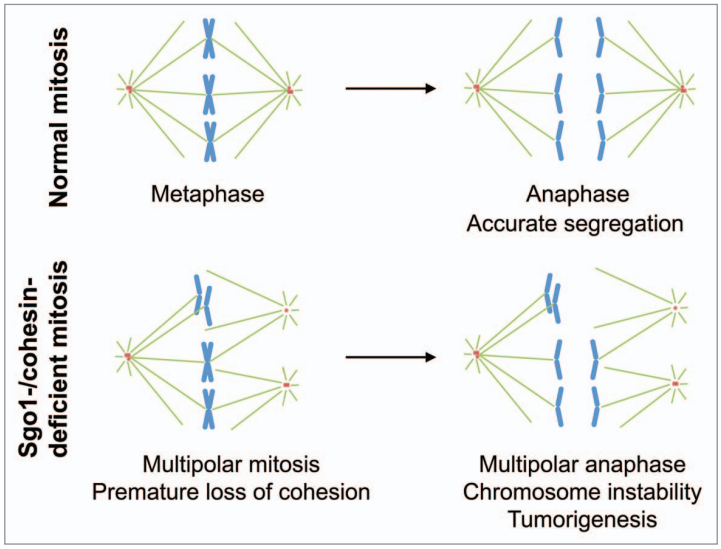

Figure 1. The chromosome and spindle abnormalities observed in Sgo1- or cohesin-deficient cells and their potential roles in generating chromosome instability.

References

1. Schvartzman JM, et al. Nat Rev Cancer 2010; 10:102-15; PMID:20094045; http://dx.doi.org/10.1038/nrc2781.
2. Barber TD, et al. Proc Natl Acad Sci U S A 2008; 105:3443-8; PMID:18299561; http://dx.doi.org/10.1073/pnas.0712384105.
3. Solomon DA, et al. Science 2011; 333:1039-43; PMID:21852505; http://dx.doi.org/10.1126/science.1203619.
4. Tang Z, et al. Dev Cell 2006; 10:575-85; PMID:16580887; http://dx.doi.org/10.1016/j.devcel.2006.03.010.

5. Kitajima TS, et al. Nature 2006; 441:46-52; PMID:16541025; http://dx.doi.org/10.1038/nature04663.
6. Yamada HY, et al. Cell Cycle 2012; 11: In press; PMID:22262168.
7. Wang X, et al. Dev Cell 2008; 14:331-41; PMID:18331714; http://dx.doi.org/10.1016/j.devcel.2007.12.007.
8. Beauchene NA, et al. Cell Cycle 2010; 9:1774-80; PMID:20404533; http://dx.doi.org/10.4161/cc.9.9.11524.
9. Schöckel L, et al. Nat Cell Biol 2011; 13:966-72; PMID:21743463; http://dx.doi.org/10.1038/ncb2280.