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Polyploidy and dysregulated ROS signaling

The school of hard Nox?

One of the major theories put forth to explain the age-related general decline oforgan systems and increased incidence of chronic disease is the free radical theory ofaging proposed by Harman in 1956.[1] Harman proposed that reactive oxygen species (ROS) cause damage over time to the biological building blocks: DNA, proteins, and lipids leading to senescence, cell death, disease and ultimately death of the organism. However this explanation of aging and age-related disease has not proven fruitful in terms of therapeutics, as general antioxidant therapy has largely failed to prevent agerelated disease. Thus, it has become increasingly clear that there is much that we do not understand about the biological function(s) of ROS. This gap in knowledge highlights the importance of understanding how specific ROS producing enzymes function and how these enzymes become dysregulated as a means to insight into the role that ROS play in the aging process. In the current issue of *Cell Cycle*, McCrann and colleagues[2] have provided an important link between aging and ROS by demonstrating that increased expression of an ROS-producing enzyme, Nox4, results in vascular smooth muscle cell (VSMC) polyploidy, a common aging-related phenomenon. In particular, McCrann and colleagues document that aging rats have increased endogenous Nox4 expression that correlates with increased polyploidy—a process whereby normally diploid cells with two sets of homologous chromosomes (2N) acquire multiple genome copies up to 128N. These findings are important as polyploidization has previously been linked to aging and chronic disease. The study by McCrann and colleagues is also important as they established that Nox4 was more abundant in isolated polyploid cells than in normal diploid cells. Of course, the important question is whether Nox4 is sufficient to drive polyploidization. To their credit, McCrann and colleagues found a causal role for Nox4 as forced overexpression of Nox4 in cultured VSMCs readily produced polyploidy. These findings are consistent with a recent report describing an override of the spindle checkpoint by oxidative stress, leading to aneuploidy, or the presence of an abnormal chromosome set.[3] Since changes in ploidy are typically permanent, the notion that ROS increase ploidy provides an attractive explanation why antioxidant therapies have proven ineffective in reversing age-related phenomena.

The NADPH oxidases (Noxs) are a family of highly conserved enzymes involved in the production of ROS. There are seven known isoforms of this family that share a basic structure that facilitates electron transfer across membranes to support ROS production. Among these isoforms, Nox4 has been found in vascular cells such as endothelial cells, smooth muscle cells and, in contrast to other Nox isoforms, is thought to principally produce peroxide as its ROS. Moreover, Nox4 is particularly appropriate amongst Nox isoforms to support age-related pathology as it is constitutively active. Thus, the increased Nox4 expression described by McCrann and colleagues would be expected to be sufficient for increased steady-state levels of ROS in the aorta and, as a consequence, increased ploidy.

Another important feature of the study by McCrann et al. is the demonstrated link between Nox4 and survivin expression. Survivin is a mitotic regulator involved in suppression of apoptosis and regulation of cell division. Survivin expression correlates with aggressive tumors and is linked to poor prognoses, whereas decreased survivin expression facilitates polyploidy.[4] Thus it was of particular interest that Nox4 overexpression resulted in downregulation of survivin mRNA, providing a mechanism for Nox4-mediated polyplodiziation. These observations suggest that one manifestation of "dysregulated" ROS signaling may be downregulation of survival signaling and, as a consequence, an emergence of age-related pathology.

In summary, the results reported by McCrann and colleagues provide new insights into the role(s) of ROS in age-related disease. Their findings suggest that chronic increases in ambient ROS have the potential to produce irreversible changes in gene regulation (through ploidy) that would not be expected to respond to antioxidant therapy. Their findings reinforce the notion that further work identifying the distinction between physiologic and pathophysiologic ROS signaling is likely to provide key insights into chronic diseases and age-related phenomena.

Acknowledgements

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The long and short of it

A new isoform of TIN2 in the nuclear matrix

The ends of linear chromosomes require special mechanisms for their replication and protection. These mechanisms are mediated by telomeres, the specialized nucleoprotein complexes at chromosome ends. Mammalian telomeres are comprised of TTAGGG repeats that end in a 3′ single stranded overhang and shelterin, a multisubunit complex that controls telomere maintenance by regulating access to telomerase and that protects chromosome ends by sheltering them from the DNA damage response. Shelterin contains six subunits: two double-stranded DNA binding proteins that regulate telomere length and chromosome end protection, TRF1 and TRF2, respectively; one single-stranded DNA binding protein that binds to the 3′ overhang, POT1; and three others, TIN2, TPP1 and RAP1.

TIN2 is distinguished by its central position in the shelterin complex. TIN2 was initially identified by Campisi and colleagues in a two-hybrid screen with TRF1 and shown to function in telomere length regulation.[1] Scrutiny of its primary structure revealed a small, unremarkable protein of 354 amino acids, whose only distinguishing feature was its TRF1 binding site. However, subsequent studies revealed multiple protein binding sites and functions for TIN2. In addition to binding to TRF1, TIN2 binds to TRF2 (thereby linking telomere length regulation and chromosome end protection)[2-5] and to TPP1, which binds to POT1 (thereby linking the double stranded telomeric DNA to the single stranded 3′ overhang).[6,7] TIN2 is essential for the stability of the shelterin complex and expression of TIN2 mutant proteins has dramatic consequences for telomere length maintenance and chromosome end protection.[1,3,5] TIN2 has also been implicated in sister telomere cohesion. TIN2 (along with TRF1) binds to the SA1 ortholog of the SCC3 cohesin subunit8 and TIN2 regulates tankyrase 1,[9] a poly(ADP-ribose) polymerase that is required for sister telomere separation at mitosis.[10]

How does TIN2 regulate all these activities? Well, it may not be acting alone. In this issue of *Cell Cycle*, Campisi and colleagues report on a novel form of TIN2.[11] Upon analysis of TIN2 protein sequences in the database, the Campisi group noticed that there were two distinct human TIN2 proteins: the original one that they discovered, of 354 amino acids (now termed TIN2S for short) and a second one that is identical to TIN2S, but contains an additional 97 amino acids at its carboxy terminus (termed TIN2L for long). In this report they show that the short form likely results from alternative splicing leading to retention of an intron that introduces a translational stop codon

after amino acid 354. Immunoblot analysis of several human cell lines showed that these two TIN2 isoforms are similarly expressed, however TIN2L is only detected when cells are lysed under harsh denaturing conditions, explaining why it was not noticed previously. Indeed, they show that TIN2L, but not TIN2S, is tightly bound to the nuclear matrix. Previous studies have shown that telomeres are anchored to an insoluble structure within the nucleus[12] and thus, TIN2L could serve as a tether, anchoring telomeres to the nuclear matrix.

What are the functional implications of this new major isoform of TIN2? TIN2L (like TIN2S) contains binding sites for TRF1, TRF2 and TPP1. And indeed, the Campisi group shows that TIN2L (at least when overexpressed) can bind to TRF1 and TRF2. Thus, there may be discrete complexes of shelterin containing either TIN2S or TIN2L. The composition of such complexes remains to be determined. Perhaps the other shelterin subunits (TPP1, POT1 or RAP1) are preferentially associated with one TIN2 isoform or the other. Association might be regulated during the cell cycle. The presence of TIN2L would distinguish the complex by tethering it to the nuclear matrix. Moreover TIN2L with its unique 97 amino acid tail might differentially recruit (yet to be identified) proteins to the complex. A crucial question for the future is whether one or both of the TIN2 isoforms is required for the varied functions of TIN2 in telomere structure, replication, protection, and cohesion. The similarity of the transcripts hampered the group's ability to selectively reduce one isoform but not the other with shRNA. Hence, future experiments will require combining depletion of both forms with exogenous expression of one or the other. And finally, if there was not already sufficient motivation to understand how TIN2 works, the recent identification of TIN2 mutations in the human bone marrow failure syndrome Dyskeratosis Congenita,[13] makes understanding TIN2 more worthwhile than ever.

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Killing metastasis with engineered bacteria

The study by Hayashi et al.[1] presents a highly novel and promising new approach to employing intracellular lytic bacteria for cancer therapy. There have been previous attempts to develop bacterial-based therapies with limited success.[2] The genetically-engineered auxotrophic Salmonella bacteria employed by Hayashi et al. have actually cured some malignancies in mice and show promise for future development.[3-5]

An indisputable glory of modern biological science has been the understanding and effective treatment of disease producing microorganisms. Their significantly different biochemistry and structure often affords unambiguous targets for selective chemotherapy. We have experienced a golden age of antibiosis in which simple compounds, isolated from organisms or produced by synthetic chemistry, very often afforded spectacular results. The offending organisms could be eradicated with minor damage to the patient.

The search for a chemical "silver bullet" has been far less successful against the conundrum posed by cancer. Since malignant cells arise from patient tissue and, although clearly abnormal, only rarely afford biochemical differences that can offer a target for selective eradication of diseased cells. Nevertheless, many tumor cells manifested significant sensitivities to chemotherapeutic agents. Through a long and difficult slog, chemical agents were developed that, depending on circumstance, could be effective in reducing or even curing a malignancy. However, effective therapies are rigorous in the extreme and many tumors remain beyond the reach of current art.[6]

Tumor cells can display a variety of characteristics radically different from normal. They are disorganized, both chemically and structurally. They have lost the response to many significant controls including chemical and architectural signals. Most often, chromosomes are manifestly scrambled. By virtue of their aberrant growth, they no longer have appropriate blood and lymphatic supplies. They grow relatively slowly but never stop. Most appear unhealthy and have a greater sensitivity to chemotherapeutic agents. However, their regulatory breakdown and disorganization unfortunately can confer tumor cell survival. They can evolve resistance to therapeutic drugs and, what is most threatening, develop metastases.[6]

A nearly universal characteristic of tumor cells is their metabolic and structural disorganization. Might it be possible to develop a smart therapeutic agent that could distinguish such aberrant cells from normal and destroy them? The paper of Hayashi et al.[1] suggests that this indeed may be possible. Tumor cells usually have aberrant levels of amino acids due to loss of regulation, degradation of necrotic material, etc. Hayashi et al.[1] use a suitably-engineered version of *Salmonella typhimurium*. Normally a notoriously destructive disease agent, the auxotrophic strain described by Hayashi et al. is restricted to growth in the rich nutrient milieu of a tumor interior, but cannot multiply in the cells or fluids of healthy tissue.[3-5]

The use of bacteria as cancer therapeutic agents has a long and not overly promising history. Anecdotal evidence reported that severe bacterial infections could result in the occasional remission.[7] Whether this was due to a direct effect of bacteria on tumor cells or a secondary effect of the stimulation of human response or some other cause was unknown. More recently there have been focused efforts using obligate anaerobes which could destroy necrotic cells but could not, by themselves, affect significant cures.[2] The bacteria described by Hayashi et al.[1] depend instead on the uniquely rich milieu of tumor cells to affect their selected toxic effect. In essence, the agent, *S. typhimurium*, developed by nature over millennia, has been corralled into a very selective therapeutic action. Hayashi et al.[1] report the eradication of tumor cells using their engineered *S. typhimurium*. Now that is surely a very smart agent!

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