Research Roundup

How chronic stress exacerbates cancer

Having cancer is stressful enough without the knowledge that stress itself promotes tumor growth. But Premal Thaker, Liz Han, Aparna Kamat, Anil Sood (University of Texas M.D. Anderson Cancer Center, Houston, TX), and colleagues have found just that: human ovarian carcinoma cells injected into mice form tumors that grow more when the mice are exposed to chronic stress. Drugs that block stress responses may therefore be appropriate for certain cancer patients.

Humans and mice have two major responses to stress. The adrenal gland responds by producing glucocorticoids, and the sympathetic nervous system by producing catecholamines, which bind to the β adrenergic receptors ADRB1 and ADRB2. The Texan group identified a pathway leading from ADRB2 to protein kinase A (PKA) and the production of VEGF. VEGF is known to induce angiogenesis (the growth of new blood vessels) and thus to aid tumor growth.

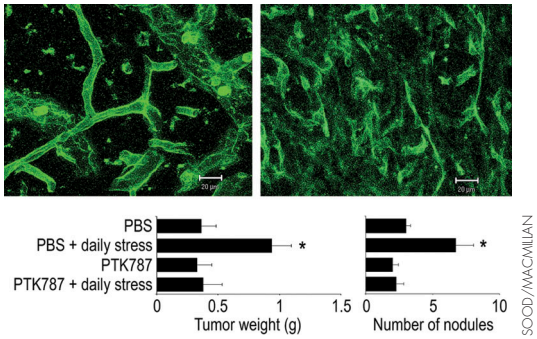
The group had earlier found that patients with greater distress had higher levels of VEGF, and that catecholamines but not glucocorticoids were clearly linked in vitro to increased production of VEGF by cancer cells.

In the current study with the injected mice, they found that social isolation, 2 hours of immobilization daily, or a β_2 agonist caused tumor nodule number and mean tumor weight to approximately double or triple. Levels of VEGF and mean vessel density in the tumors were both increased. All of these stress-mediated increases were blocked by reducing the levels of ADRB2 (but not ADRB1) or by antagonizing PKA or VEGF.

Only tumors that express ADRB2 will be susceptible to this particular stress pathway. It is not clear how large this universe is, as there have not been large-scale surveys of ADRB2 expression in different tumor types.

Patients with tumors that express high levels of ADRB2, and who have significant stressors such as surgery or lack of social support, would be the best candidates for a trial of β blockers as potential cancer therapeutics. These adrenergic receptor antagonists are widely used for hypertension, but may find a new application in reducing the negative effects of stress on tumor growth. JCB

Reference: Thaker, P.H., et al. 2006. *Nat. Med.* doi:10.1038/nm1447.



Stress-dependent increases in vessel density (top, left to right) and tumor weight and number (bottom) are prevented by VEGF antagonist PTK787.

Charged signaling

Areas of negative charge come and go on the plasma membrane, report Tony Yeung, Sergio Grinstein (Hospital for Sick Children, Toronto, Canada), and colleagues. Loss of the charged areas correlates with the loss of certain signaling proteins from the membrane and inactivation of related downstream activity.

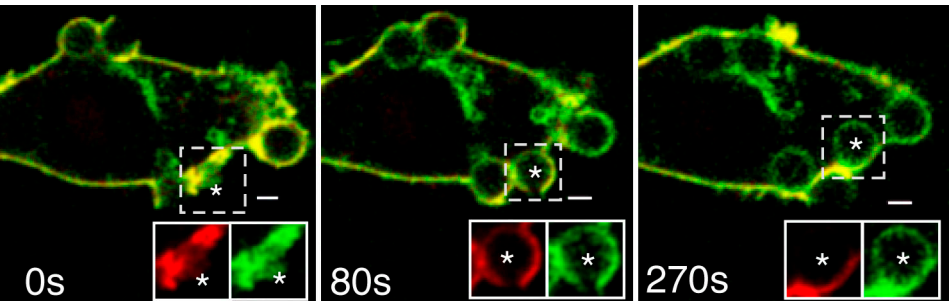
Attraction based on charge is well established for sequence-specific protein–protein interactions. But the Toronto group documented an electrostatic switch that was evident with many different probes, with many different anionic lipids, and on liposomes that lacked any proteins. The probes were modeled on fragments of the K-Ras protein and combined polycationic sequences with a hydrophobic anchor to keep the probe in the plasma membrane rather than interacting with the anionic nucleoplasm.

The probes were displaced from the intracellular face of the plasma membrane by three different conditions: an influx of calcium; flipping out of negatively charged phosphatidylserine (PS); and the later stages of phagocytosis. Rac1 dissociated from phagosomes with similar kinetics, even if locked in a GTP-bound form.

During phagocytosis, signaling results in hydrolysis of negatively charged membrane-localized PIP2 to form uncharged diacylglycerol and cytoplasmic IP3. Inhibition of PIP2 hydrolysis reduced the loss of the probes from phagosomes.

The Toronto group suggests that charge-dominated attachment may be reversed downstream of calcium elevation, PS flipping, and PIP2 hydrolysis for a wide range of biological pathways. "If it is happening in other contexts [such as around individual receptor kinases] it would be much harder to tell," says Grinstein, because of the smaller surface areas involved. But Grinstein will be looking next at endocytosis, based on evidence that endosomes and plasma membranes differ in their charge. JCB

Reference: Yeung, T., et al. 2006. *Science.* 313:347–351.



A probe detecting surface charge (red) is depleted during phagocytosis.

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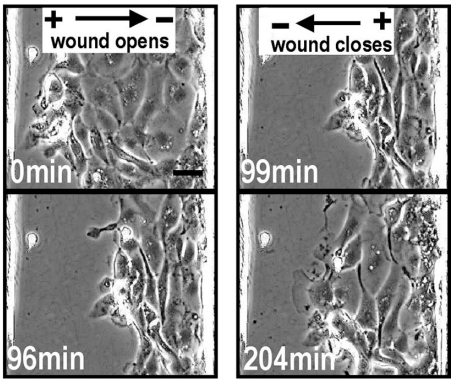
Electric healing

Wounds have electrical fields that help cells to flood into and heal them, according to Min Zhao (University of Aberdeen, UK), Josef Penninger (Institute of Molecular Biotechnology, Vienna, Austria), and colleagues.

For over a decade, Zhao has been studying how cells migrate in response to electrical fields. It has been a lonely field, however. Electricity does not fit easily into the gene–protein paradigm of cellular control, and the field's reputation was tainted by some poorly controlled experiments conducted early in the 20th century.



PENNINGER/MACMILLAN

Electrical fields can both open (left) and close (right) a wound.

Now, Zhao and colleagues have confirmed claims first made more than 150 years ago that wounds generate electrical fields. There is normally a potential difference between basal tissue layers and apical skin surface—a difference generated by transport of Cl^- ions outwards and Na^+ ions inwards. But this potential difference is short circuited by a wound. The wounded basal edge becomes electrically more like an apical surface, so that now the potential difference is between this damaged basal edge and the undamaged, internal basal tissue. The result is an electrical field directed into the wound.

The researchers found that electrical fields of this magnitude could direct cell migration both in vitro and in vivo, either slowing or accelerating wound healing, depending on the field directionality. The correlation between the magnitudes of naturally occurring and experimentally effective field potentials "got us more and more excited and thinking this is a real phenomenon," says Zhao.

The pathway required PI3Kγ and was enhanced by loss of PTEN. These proteins are well known as mediators of chemotactic signaling, but with a whole genome screen the group hopes to discover molecules unique to electrotaxis. JCB

Reference: Zhao, M., et al. 2006. *Nature.* 442:457–460.

Relaxing after damage

Phosphorylated KAP-1 fans out from DNA damage sites, spreading a message of chromatin relaxation, according to Yael Ziv, Yosef Shiloh (Tel Aviv University, Tel Aviv, Israel), and colleagues. The temporary relaxed state may allow proteins that detect and repair damage to gain better access to DNA.

Chromatin relaxation after DNA damage has been seen before but has been primarily a local effect at the site of damage. The Israeli group, however, documented a global effect that increased susceptibility to nuclease digestion throughout the genome.

The relaxation begins with the damage-detecting ATM kinase. The researchers found that ATM phosphorylates KAP-1, previously known as a transcription corepressor, and that this phosphorylated form spreads within minutes from the damage sites to a pan-nuclear localization. The result is a transient chromatin relaxation that lasts an hour or so. KAP-1 lacking the critical phosphorylation site does not induce relaxation.

The phosphorylated KAP-1 "is carrying a message to the chromatin," says Shiloh. "If we had used 10 or 11 minutes as our first timepoint [it would have spread already and] we would have lost a critical element of this story."

The group is now looking for proteins that interact with KAP-1 only before or only after phosphorylation, and for proteins or modifications that define the relaxed DNA state. The effect of that relaxed state is unknown; one possibility is that it helps the transcriptional apparatus to scan DNA for further damage. JCB

Reference: Ziv, Y., et al. 2006. *Nat. Cell Biol.* doi:10.1038/ncb1446.

Making a single centrosome

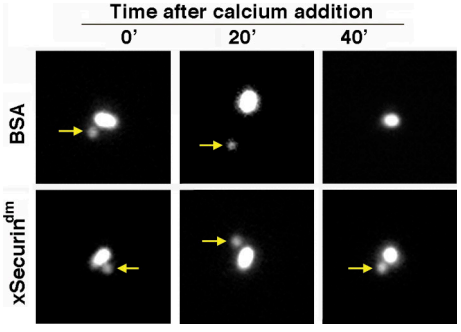
Separase cuts sister chromosomes apart at the end of mitosis. The same enzyme also, say Meng-Fu Bryan Tsou and Tim Stearns (Stanford University, Stanford, CA), releases a block to centriole and thus centrosome duplication. "It's so simple to have separase involved in both processes, because it is so critical to not do either one prematurely," says Stearns. "It does make perfect sense that it is arranged this way."

Microtubules can focus to form an organizing center in several ways, but "in dividing cells, the centrosome is the main player," says Stearns. "And if you control centriole number you've controlled centrosome number."

His group found recently that there is a block to reduplication that is intrinsic to centrosomes rather than being determined by the cytoplasm surrounding them. This block is now found to be released not by mitotic exit or by G1 kinase activity but by separase activity.

The separase disengages each tightly apposed pair of centrioles—a process that is subtle in cultured cells but more obvious in frog extracts where there is no G1 phase. During the subsequent cell cycle, a new centriole then forms orthogonal to each of the two disengaged centrioles. The visible fibers that connect daughter centrioles may contain a separase substrate, but there are no obvious candidates as yet. JCB

Reference: Tsou, M.B., and T. Stearns. 2006. *Nature.* doi:10.1038/nature04985.



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Daughter centrioles separate (top) unless separase is inhibited (bottom).