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edited by Rod Capaldi (rcapaldi@uoregon.edu)

THE ECTOPIC ATP SYNTHASE: MORE FUNCTIONS, INVOLVEMENT IN MORE DISEASES, AND NOW A KEY TARGET IN DRUG DISCOVERY AND DIAGNOSTICS

Hopefully many readers of this edition will have read September's MitoAlmanac and be aware that the ATP synthase, once thought to be a solely mitochondrial enzyme, is also present on the plasma membrane of many cells. If you read the previous edition you will also know that the ectopic ATP synthase is the HDL receptor and likely plays an important role in Alzheimer's disease.

Further studies of the role of this enzyme in cholesterol metabolism have been reported in the last month. Genoux et al have now shown that the ATP synthase inhibitor protein IF1 is present in the systemic circulation of humans. Using immunoprecipitation and mass spectrometry, they detected the presence of this protein in plasma, and further, showed that the levels of IF1 in individuals were positively correlated with HDL-cholesterol and negatively correlated with triglycerides. GENOUX et al. PLOS ONE 6. ISSUE 9 e23949 (2011).

BUT WAIT THERE'S MORE! THE ECTOPIC ATP SYNTHASE IS INVOLVED IN VASCULAR TONE AND BLOOD PRESSURE CONTROL.

CF6 (coupling factor 6) is an essential subunit of the mitochondrial ATP synthase, and by analogy although not conclusively proven, it is a subunit of the ectopic ATP synthase. Over the last decade Okumura and colleagues have provided compelling evidence that CF6 is present in blood plasma and that it is released from the surface of HUVEC cells by shear stress. They have shown that this induces vasoconstriction while the biologically active site at the C terminal portion of CF6 suppresses prostacyclin generation via inhibition of cytosolic phospholipase A2. (reviewed in OSANAI T. MAGOTA K & OKUMURA K NAUNYN-SCHMIED ARCH PHARMACOL 380. 205-14 (2009).

Earlier suggestions that CF6 was being sheared from ectopic ATP synthase have not been reiterated lately and it seems probable that there is a "free" pool of CF6 present on the surface of vascular cells, separate from that involved in the stalk part of the ATP synthase. A key target for the CF6 released by the sheer stress is the ATP synthase itself. Okumura and colleagues were able to show that CF6 binds to the beta subunit of the ectopic ATP synthase on vascular endothelial cells, resulting in an increased ATPase activity and decreased intracellular pH of these cells (OSANAI T. et al. HYPERTENSION 46. 1140-6 (2005)).

This action of CF6 leads to up-regulation of several pro-atherogenic molecules including endothelin 2, urokinase-type plasmingen activator receptor, estrogen receptor beta and lectin-like oxidized low density lipoprotein receptor 1. Of possible diagnostic significance, the plasma levels of CF6 are elevated in patients with essential hypertension.

AND MORE!

Various studies have shown that plasma levels of CF6 are greatly increased in
diabetes, LI XL. Et al. INT. J. CARDIOL. 117. 411-2 (2007);
end-stage renal disease, OSANAI T. et al. kidney int. 64. 2291-7 (2003)
acute myocardial infarction DING WH. et al. HYPERTENS RES. 27. 7-7-22 (2004);
and coronary heart disease (CHAI SB et al CIRC J. 71. 693-7 (2007)).

Not surprisingly, then, the ectopic ATP synthase has become a novel target for cardiovascular drugs as described in a recent review by FU. Y & ZHU. Y. (CURR. PHARM.DES 16. 4074-9 (2010)).

HOW MUCH EXCITEMENT CAN YOU TAKE AT ONCE? WHAT IF THE ECTOPIC ATP SYNTHASE, WAS INVOLVED IN CANCER?!

There are several hallmarks of cancer and these include altered metabolism with a strong preference of tumor cells for making ATP by glycolysis instead of by OXPHOS. Also cancer cells stimulate angiogenesis to increase blood supply to the tumor cells…and these cells must evade detection and destruction by the immune system.

THE ECTOPIC ATP SYNTHASE AS THE ANGIOSTATIN RECEPTOR.

The first evidence that an ectopic ATP synthase functioned as an angiostatin receptor in HUVEC cells was provided by Pizzo and colleagues. Angiostatin is a powerful anti-angiogenesis agent and has been studied for more than a decade as a possible anti-

cancer therapy. It inhibits cell surface ATP synthase for both hydrolysis and synthesis activity and has been shown to inhibit the ATP synthase of isolated mitochondria from beef and yeast as well as the enzyme from E. coli. This inhibition of the ectopic enzyme reduces cellular proliferation and in combination with low extracellular pH, there is intracellular acidification along with cytotoxicity (reviewed in CHI SL. & PIZZO SV. CANCER RES 66. 875-82. 2006; See also ZHAN M. et al. ACTA BIOCHIM BIOPHYS. SIN. 42. 530-7 (2010).

In collaborative studies Pizzo's group and my laboratory showed that a monoclonal antibody against the beta subunit of ATP synthase had identical effects to angiostatin and blocked the binding of this protein to the cell membrane (CHI SL. et al. CANCER RES. 67. 4716-24. 2007). Together, we also showed that the inhibitor protein (IF1) mimicked angiostatin, competed for the same binding site and blocked angiostatin effects if added first. IF1 inhibits ATPase activity but does not inhibit ATP synthesis (BURWICK NR. et al. J.BIOL. CHEM. 280. 1740-45. 2005) . Very recent work by Wang et al using a hepatoma xenograft mouse model has provided in vivo evidence of the anti-tumoral potential of anti F1beta mAbs (WANG WJ. et al. CLIN. EXP. MED. (2011) E pub ahead of print).

Pigment epithelium derive factor (PEDF) is another potent blocker of angiogenesis in vivo and of endothelial cell migration and tubule formation. It has been reported recently that this protein binds to the ectopic ATP synthase and blocks angiostatin binding. Further, PEDF inhibits ATP production in the extracellular medium, and a monoclonal antibody to the beta subunit of the ATP synthase blocks PEDF binding and this ATP production (NOTARI L. et al. FEBS J. 277. 2192-5. 2010).
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ECTOPIC ATP SYNTHASE AND EVASION OF IMMUNE RESPONSE.

Vgamma9/Vdelta2 T lymphocytes, which are known to be involved in immune response to tumor cells, have been shown to recognize the F1ATPase part of the ectopic ATP synthase complex. In an interesting set of experiments Martinez and co-workers have studied the immune function of the ATP synthase further using a novel ATP analogue Appp1 (triphosphotic acid 1-adenosin-5'-ylester 3(3-methylbut-3-enyl)ester. This compound has been found as a metabolite of cells treated with aminobisphosphonate drugs, which potently sensitize cells to Vgamma9/Vdelta2 T lymphocytes. This compound, which is spontaneously produced by cancer cells, binds the ectopic ATP synthase resulting in activation of the T lymphocytes (reviewed in VANTOUROUT P. et al. WORLD J. GASTROENTEROL. 16. 5925-35. 2010).

NEW METHODS OF MONITORING MITOCHONDRIAL STRUCTURE AND FUNCTION.

Chen et al describe a novel mitochondrial calcium fluorescent indicator by genetic means. They use the probe to monitor mitochondrial calcium changes in HeLa cells induced by different drugs (Chen M. et al. Acta Biochim. Biophys.Sin. 2011. Eprint Aug 30 ahead of pub).

A novel method for monitoring electrochemically mitochondrial metabolic activity is described by Arechederra, et al. Mouse mitochondria are "wired" to a carbon electrode surface to reroute electrons before they can reach cytochrome c oxidase. Direct measurement of electrical current and membrane potential are made during the oxidation of pyruvate and fatty acids as substrates and the effects of mitochondrial inhibitors on

such measurements are described (Arechederra RL. Waheed.A. Sly. WS & Minteer SD. Analyst 136. 3747-52. 2011).

THE PIONEERS OF MITOCHONRIAL RESEARCH REMEMBERED. 2). PETER MITCHELL

Let me provide you a fairy tale. Once upon a time, not long ago (by my standards), there was a man who liked biology and worked at one of the premier universities in England but became disillusioned with the academic life as practiced at such institutions. So, he bought an old run down house in the middle of nowhere (Bodmin, Cornwall) which he made his home and in which he built a laboratory. From there he set out to prove a revolutionary idea he had about energy transduction, using his own money and donations to fund the research. For many, many years other researchers in the field were rude and nasty to our hero and even had meetings to plot common strategies to discredit his ideas. But slowly, the evidence amassed, and our hero won out and was celebrated all over the Queen-dom and the world more widely.

Detailed evidence that the above is not a fairy tale is provided in a beautifully written book "Peter Mitchell and the Vital Force" by Milton Saier who sums up Mitchell as follows; "he was the brilliant but apparently nutty intellectual dynamo who nearly single-handedly provided conceptual advances which exceeded those of almost any other biologist, living or dead. He revolutionized our ideas about how cells couple oxidative energy-releasing reactions to the synthesis of chemical energy in the form of ATP. He represents to vectorial molecular biology what Einstein represents to nuclear physics and Fischer represents to organic chemistry".

There are several brief reviews of Mitchell and his work that are well worth reading. The one I recommend is by Peter Rich who took over from Mitchell as Director of the Glynn Institute (A PERSPECTIVE ON PETER MITCHELL AND THE CHEMIOSMOTIC THEORY. RICH P.R J. BIOENERG. BIOMEMBR. 40. 407-10. 2008).

Peter Rich kindly provided a couple of stories about Mitchell, the man, below;

Peter had a wide range of interests outside science. For example, he had a keen interest in economics, often traveling to London to deal with his investments in what he called a 'glorified day at the races'. This interest extended to direct practices at Glynn where, at one time, he minted his own silver 'Glynns' and even offered them to sometimes bemused staff in lieu of their cash salaries on the basis that they would keep up with, or beat, inflation (which sadly, and dramatically, did not live up to expectation). He also introduced a system that adjusted salaries each month to take into account inflation; a great system for staff but a nightmare for administration. His advice and personal practice on pensions were rather insightful given the present economic climate – instead of formal pension schemes he instead recommended buying works of art and antiques that could be stored and resold in retirement.

Peter had a great, rather mischievous, sense of humor. For example, he was fond of recounting his tale of a meeting of Nobel Laureates with the Queen, where he took one of his silver Glynns to show her. When his turn came in line to meet Her Majesty, he proudly showed her the Glynn, which to his horror, she took, put into her handbag and moved on.

This humor appeared in many other ways. He had a lifelong hearing problem and could often be seen readjusting his hearing

aid at conferences. I commented one time about how inconvenient this must have been for him and he answered ‘but I can also tune out instead of in’. On another occasion, we were returning together from a magnificent dinner, hosted by a drug company, which extended over very many courses and hours. Peter’s summarising comment whilst we were returning to the hotel very late at night and exhausted was that he had ‘never before been subjected to so much hospitality’.