SEATTLE PROSTATE INSTITUTE

PCa Commentary

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Your comments and requests for information on a specific topic are welcome at ecweber@nwlink.com

!!! PCa Now on the Web

Beginning this month, the content of PCa Commentary, past and future, will be available online at www.seattleprostate.com, the website of the Seattle Prostate Institute. From the SPI home page, just click on "Physician Education & Training" and then on "PCa Commentary".

For your convenience, the content pieces will be indexed under the major topic headings shown below. Click on a particular topic and a list of related article titles will appear. Each title links to the full article text.

It is my hope that this new online feature will be a useful resource to our readers.

Ed Weber, M.D. - Editor

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BASIC SCIENCE & BIOLOGY: Gene Silencing Revisited – Now a Hot Item

Gene silencing was recently featured in a Seattle Times story, a reminder to clinicians that we will have to wrap our minds around these new concepts in order to be well informed. Developments arising from basic molecular biology are moving to center stage. However, before reporting the news, let me first offer my simplistic primer about the basic biology of this area of DNA genetic infomatics. My brief review begins with a mental cartoon describing an activated cellular receptor traveling into the cell's nucleus and heading toward DNA, at which point it finds it has to penetrate a fuzzy molecular barrier created by the histones, the "spools" around which the DNA is wrapped. An access "cleft" must be created, and is achieved by the insertion of small molecular "props" (acetyl groups) that push apart the tight bonds of the barrier, thus facilitating the receptor's passage. (Conversely, a open barrier may be closed by removing the acetyl groups, a process termed "de-acetylation".) Once past the barrier, the business end of the receptor (with all its helpers: the coactivators, corepressors and the transcription assembly) must find free access to the DNA target, the promotor site where a gene's transcription begins. Another possible obstacle may frustrate successful access to DNA. If a methyl group "sits on top" and preempts access to the promotor site, the proper "fit" of the receptor complex and the DNA is prevented. No DNA contact, no transcription. This "methylation" is termed "epi-genetic", literally "above the genome", and can also be termed "imprinting", and is a common method of regulating gene expression. (The Roche pharmaceutical company must think this area of biology is important since it recently invested $100,000,000 in the Seattle-Berlin biotect company, "Epigenetics," to pursue potential therapeutic developments.) If the methyl group is removed then transcription can begin. What to methylate; what to deacetylate ?! It remains a fascinating unsolved mystery how the cell "knows" how and when to do these things... So much for the primer. Now on to the news.

The recent Seattle Times article highlighted a new test that utilizes the identification of methylated genes as a marker of the risk of developing colon cancer. The basis for the news was the study published in SCIENCE, March 14, 2003, "Loss of IGF2 [Insulin-like Growth Factor] Imprinting [methylation]: A Potential Marker of Colorectal Cancer Risk." Humans inherit two copies (alleles) of each gene - one maternal, one paternal. The gene of interest here is IGF2, an important gene for many of our internal systems which stimulates proliferation and activity of its targets. In the normal state we receive one of two alleles for this gene methylated, hence silenced. The test assesses the extent of methylation of these two alleles, and if **both are unmethylated,** and therefore both functional, that individual gets an extra boost of growth factor, thereby raising the risk of developing colon cancer to 5 times normal ! A converse example can be found in uterine endometrial cancer where the invasiveness of tumor is enhanced by unwanted methylation of a "tumor suppressor" gene, E-cadherin, that normally serves to inhibit migration of tumor cells away from the primary site (CANCER 2003;97:1002).

Returning the discussion to methylation in prostate cancer, I'll report the most recent article by Drs. Sidransky and Epstein (J UROL 2003, March) in which they follow-up on their earlier work [discussed in March PCa Commentary] utilizing a quantative test for methylation of the gene, GSTP1, which codes for the protective cellular antioxidant, glutathione. In the new work they tested a single core from each of 29 prostate biopsy sets. These cases were considered problematic and non-diagnostic by the initial pathologists. By using the methylation marker they were able to diagnose cancer in 11 of 15 cores. Four cores had a single foci of cancer of only 1 mm. and 7 had 0.05 mm. foci. The Gleason sum for each cancer case was < 6. On full review, fourteen additional specimens were benign and none of these showed methylation. Clearly the argument is being made in support of this technology for diagnosis, especially confirmatory information in difficult cases.

When it becomes clear that unwanted methylation can cause adverse effects, it's natural to speculated whether therapy could be developed that would de-methylate silenced genes whose function is

*desirabl*e. Perhaps a drug that demethylates the GTSP1 prostate gene might serve as treatment to avert progression of very early prostate cancer. An example of such a medication exists [5-azacytidine] and has been tried in a hematologic malignancy, but the drug is quite toxic and only is suitable to intravenous administration. A newly developed agent suitable for oral administration was report in the JOURNAL of the NATIONAL CANCER INSTITUTE, March, 2003 - "Inhibition of DNA Methylation and Reactivation of Silenced Genes by Zebularine". The study was conducted in mice and demonstrated successful demethylation . Most exciting to the researchers, zebularine slowed the growth of tumors compared to tumors in the control animals.

Caution is in order, however, when intervention is aimed at this basic level of the operating systems of cellular function. A brief communication in the February 19, 2003 issue of the JNCI reported that when tested in tissue culture using pancreatic cancer cells, the demethylating agent 5-azacytidine *reversed* the methylted silence of several tumor suppressor genes thereby *promoting* aggressive tumor growth. Clearly very careful studies will be required before drugs of this type are approved for human use. It makes one recall the old adage - "Be careful what you wish for, you might just ...."

Bottom Line: Tests of gene methylation are promising for diagnosis and risk assessment.

DIAGNOSTICS: PAP - Making a Comeback as a Prognostic Indicator

Unless my personal experience is unusual, most clinicians managing prostate cancer have not ordered the serum prostatic acid phosphatase (PAP) test for quite a while. The authors of the paper "Long-Term Outcomes after Treatment with External Beam Radiation Therapy and Palladium 103 for Patients with High Risk Prostate Cancer" (CANCER, February 15, 2003, p. 979) have presented convincing data that is likely to change our usage. This excellent paper by Dr. Michael Dattoli (co-authored by Drs. Larry Wood and Kent Wallner, both from the University of Washington) finds that a pretreatment PAP level of >2.5 ng/ml "the strongest predictor of failure" (P = 0.0001) compared to Gleason sum (P = 0.04) and PSA (P = 0.04). The normal range for PAP was up to 2.5 ng/ml. The PAP was especially useful in the prognosticly difficult group with PSA between 4 and 20 ng/ml. Their study involved 161 men treated between 1992 and 1996 (median follow-up: 7 years) with clinical Stage T1 - T3 PC, Gleason sum >7, and PSA level >10 ng/ml who received 41 Gy external beam radiotherapy to a limited pelvic field followed by a palladium 103 brachytherapy boost. Failure was defined as never achieving a nadir PSA of <.2 ng/ml, or rising to exceed that value. All failing patients underwent a repeat prostate biopsy and all biopsies were negative for residual PC, thereby establishing in the best possible way that the failure was due to systemic micrometastatic disease. The pretreatment PAP test will certainly have to be incorporated in the stratification of future trials and would also be informative in management of individual patients. This study derives further strength because it stands on the shoulders of several earlier studies that came to the same conclusion about the prognostic usefulness of the PAP test.

Moul (J Urol, 1998, March) found the pretreatment PAP predictive of pathologic stage and first serological recurrence in 295 men post radical prostatectomy (RP). The disease free survival at 4 years was 78.8% for men with a PAP < 3 ng/ml, compared to 38.8% for those with PAP > 3 ng/ml. The usefulness of PAP was independent of PSA value, and was particularly useful (P = 0.012) in the group with PSA > 10 ng/ml. In another study, Han (UROLOGY, 2001, April) analyzed the post RP outcome of 1681 men with clinically localized disease and found that men with a pretreatment PAP of <.4 had a 77% likelihood of freedom from biochemical recurrence at 10 years compared with 44% for those with PAP >.5. (The normal range for PAP in this study was 0 - .8 U/L.) In their study 90% of the men were clinical Stage T1C - T2 with 75% Gleason sums $\leq$7 and 95% were PSA<20.

What is PAP ? *PAP* is significantly different from *PSA*, which is an enzyme (a serine protease) secreted into the lumen of the prostatic acini by the surrounding glandular epithelium and dispatched

via ducts to enter the urethra where it serves to liquify the coagulum of the ejaculum. In contrast, the PAP is an intracellular signaling enzyme (a protein tyrosine phosphatase - a deactivator of signaling) in prostate cells and is associated with cellular differentiation. The genes for both PAP and PSA are under the control of androgen receptor signaling. One of the challenging unknowns yet to be fully deciphered is what signaling mechanisms account for the continued production of PAP and secretion of PSA in the PC cells in "androgen insensitive" prostate cancer when signaling via the androgen receptor continues independent of testosterone stimulation.

In seeming paradox to the clinical usefulness of the PAP test wherein a high value predicts for treatment failure, the PAP content in malignant prostate cells is ***less than*** in their benign counterparts. The comparatively lower PAP content in malignant cells may be consistent with our knowledge that a *high* content of PAP inhibits a prostate cell's response to the proliferative stimulus from growth factors emanating from the surrounding support cells. There is strong evidence that in the androgen deprived state, one source of stimulation to the androgen receptor comes from growth factors working through the ErbB-2 receptor on the PC cell. The resulting implication is that when cellular PAP content is high, as in the benign state, cellular sensitivity to growth stimulation signaling is *reduced.* The converse of this inverse relationship may be to allow growth stimulation to be more effective in the malignant cell. There is much yet to be learned about the cellular function of prostatic acid phosptatase, however, these several studies have shown the PAP test has useful practical applications.

Bottom Line: The PAP test has returned to a useful place in the pretreatment evaluation of outcome for primary treatment of prostate cancer.

HORMONE INTERVENTION: Ketoconazole: Further thoughts about this second-line hormone intervention

Ketoconazole is one of the triad of second-line hormone interventions to be considered when the PSA rises after successful suppression by androgen deprivation. An excellent summary of "Secondary Hormone Therapies in the Treatment of Prostate Cancer" was presented by Wm. Oh in UROLOGY Vol.60, Supplement to September 2002, p. 87. There continues to be a bit of a mystery about how ketoconazole, customarily prescribed as an "anti-fungal" agent, actually functions as a treatment for prostate cancer. However, the most common inference is that, after being exposed for a time to the low testosterone environment fostered by an LHRH agonist, the androgen receptor responds by increasing its sensitivity to testosterone. Ketoconazole is known to decrease the production of testosterone and cortisol by the adrenal gland, which normally provides about 5% - 10% of the total serum testosterone. The coupling of these two thoughts leads to speculation that this additional lowering of serum testosterone by ketoconazole may further inhibit signaling via the androgen receptor and thereby slow the proliferation of cancer cells. New information, however, suggests that this scenario may be too simplistic.

But first, what does the established record tell about the effectiveness of this drug in "hormone refractory" PC ? Because, ketoconazole may lower the adrenal's output of cortisol, replacement doses of prednisone or hydrocortisone are customarily prescribed. But the companion use of a cortisol introduces difficulty in identifying the specific independent efficacy of ketoconazole, since prednisone or Decadron themselves have significant efficacy in PC treatment.

Wm. Oh summarized the best current studies of ketoconazole *and* hydrocortisone. When efficacy was judged by the criterion of achieving a >50% decline of PSA, the studies, using the conventional dose of 400 mg TID, showed responses of 27%, 40%, and 63%. An important article (to which I will return) by Harris, "Low Dose Ketoconazole with Replacement doses of Hydrocortisone [30 mg. / day] in Patients with Progressive Androgen Independent Prostate Cancer" (J UROL. Aug. 2002, p. 542)

very nicely demonstrated that a dose of 200 mg. TID is equally efficacious, yielding a response of 55% and 30 weeks median duration. The cost was less with fewer side effects.(A 200 mg. pill costs about $3.10 = about $280-300/month.) The duration of median response has generally been four to five months, with some responses strikingly longer. In Harris' study, those men who did not respond to the 200 mg. dose had no response to re-challenge with the higher dose. Side effects from ketoconazole can be troublesome: nausea, 29%, fatigue, 14%, edema, rash, and heptotoxicity, and the drug can effect a potentially dangerous rise in the blood levels of coumadin and the statin drugs. The medication should be taken on an empty stomach.

The interpretation of efficacy and duration of response in all these prior studies is made difficult because of the current tendency for clinicians to intervene with secondary hormonal therapies at an earlier point in the face of a rising PSA. Early therapeutic intervention is likely to encounter a lower tumor burden against which treatment *may* be more effective and can introduce "lead time bias" that can confound comparing time to survival endpoints. These considerations are relevant since all the quoted studies involved substantial numbers of men with well established metastatic disease and high PSAs (median of 49 ng/ml, range 6.3 - 557.8, in the Harris study). Among the 28 men in that study only 8 men were treated for progression indicated only by PSA elevation, the rest progressing with clinically evident bone and soft tissue disease. A PSA response occurred in only 33% of men with bone and/or soft tissues disease, whereas 75% (6 of 8)of men responded in the group treated for PSA rise only. Harris also discusses the intrepretive difficulties resulting from utilizing the combination of ketoconazole and cortisol. The reported efficacy of corticosteroids alone in PC treatment is 16-29% for 30-40 mg. of hydrocortisone. Nishimura (CANCER, Dec., 2000, p. 2570) reported a 62% PSA response to Decadron at 0.5-2 mg/day.

The Harris study added information that confounds our current speculation about the mode of ketoconazole's action. The output of adrenal androgens (androstenedione, DHEA, and DHEAS) was significantly lowered by the drug, ***however,*** "the degree of androgen suppression was not associated with response nor was the degree of suppression predicted by response to low dose ketoconazole." This finding points to alternative explanations for drug mechanism of action.

The basis of the action of ketoconazole is interference with the function of a very important family of enzymes, the Cytochrome P-450 enzymes. The inhibition of one member of this family accounts for preventing the conversion in the adrenal gland of cholesterol into the androgens and cortisol. There is emerging evidence that the enzyme, Cyclooxygenase-2 (COX-2) - the target of the anti-arthritic drugs, Vioxx and Celebrex , stimulates cellular proliferation and likely is associated with initiation or progression of cancer. A recent study shows that ketoconazole via a complex feedback loop inhibits the production of this sometimes harmful COX-2 enzyme. A study by Peehl (J UROL, Oct 2002) reported *anti-proliferative* effects of ketoconaszole in tissue culture that resulted from interference with P-450 enzymes. These findings are intriguing leads that may be developed into new avenues of therapy for prostate cancer.

Bottom Line: Ketoconazole has moderate effectiveness as a second-line treatment for PCa

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Erratum

In the April issue of PCa under the topic of Hormone Intervention, Casodex 150 mg was erroneously included with the regimens that lowered serum testosterone. Casodex at 150 mg daily raises serum testosterone.