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Contents

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Author's Note: I would like to extend special acknowledgment to Charles Haney, Ph.D. who has been an invaluable help in publishing PCa Commentary since the first issue in October 2002. Next month, he will leave the Seattle Prostate Institute to assume his new job as Executive Director of the King County Medical Society. However, Charles has graciously offered to continue to edit these commentaries. Thank you, Charles.

BIOLOGY — The Very, Very Long and Short of It: A Brief History of Androgen Sensitive Prostate Cancer Cells

Early cancer is really never "early" when considered in the context of biologic history. There considerable consensus that 30 volume doubling times (26 if the tumor is half stroma) are required for a single cell to achieve a volume of one cubic centimeter ("On the Growth Rates of Human Malignant Tumors: Implication for Medical Decision Making, Friberg in J of Surg Oncol, 1997;65:284-297). And once the tumor attains a size that can be objectively detected on imaging studies further growth follows a smooth curve of exponential volume increase that

translates to linear on semilog presentation. Although the rate of growth is unique for each heterogeneous tumor mass, if a doubling time (DT) were figured at 150 days (a representative possibility for breast cancer) the time from tumor inception to one cm3 (one billion cells) would be 12 years. Calculated at that rate, to grow to the first 2 mm mass (10 M cells) it would take 8 years. Most prostate cancers grow more slowly, placing their inception at 10, 20 or 30 years before clinical detection.

The biologic growth details describing the period at the earliest cancer stages, well before clinical detection, have been studied by Berges at Johns Hopkins ("Implication of cell kinetic changes during the progression of human prostatic cancer", Clin Cancer Res 1995, May;1(5):473-80). They made cell culture measurements of the balance between the daily percentage of cells proliferating and dying in normal and premalignant prostate cells, and in malignant cells in the prostate, lymph nodes and bone. The normal cells showed a balanced rate of proliferation and death at the very low and balanced rate of <.2%/day. Criticism has been made of such cell culture studies because of the artificiality of the environment, lacking as it does, for example, the paracrine influence of nearby stromal cells. But the observations are none the less generally instructive. In their studies the glandular cells were replaced every 500 +/- 79 days. The transition to HGPIN was associated with increased proliferation compared to cell death leading to a mass doubling time of 154 days. Later in HGPIN the rate of cell death increased to match the proliferation rate leading to a steady state with, however, a 6 fold increase in cell turnover (DT 56 days). With an increase in cell turnover rate there is a greater chance for mutations to occur. The transition into localized cancer involved no further increase in proliferation but instead was associated with a decrease in the cell death rate, leading to a net continuous growth with a prolongation of the mean DT to $\geq$ 475 days. PC growth in lymph nodes and bone showed comparative increased proliferation and further reduction of cell death yielding a DT of 33 days and 54 days, respectively. No further increase in proliferation was observed in andgrogen insensitive cells from lymph node and bone, but, rather, a increase in the death rate in these androgen independent cells leading to a slowing of growth to DT 126 and 94 days. The remarkably slow proliferation rate of prostate cancer (i.e <3%/day) may explain the relative insensitivity of PC cells to chemotherapy and the lengthy gestation time (possibly 30 years) to pathologically detectable metastatic disease.

Viewed from another perspective, the growth rate of tumors can be inferred from measurements of the DT of PSA, and many analyses have been reported based on sequential observations in patients during "watchful waiting". Each individuals' PSA relationship to his tumor volume is unique, influenced by prostate size and the relative proportion of BPH to cancer (BPH secretes PSA at 1/12 the rate of PC), the tumor histology (PSA secretion decreases with increasing tumor grade, but growth rate increases), and the amount of the neuroendocrine component (which secretes chromogranin, but not PSA), and, of course, other unknown individual biologic variables. In the 12 studies I have reviewed it was not surprising that the major generalization is the wide variability in PSA doubling times. The width of this spectrum makes calculating a "mean PC DT" mathematically interesting, but biologically rather irrelevant. For example, Choo reported a study from University of Toronto in which watchful waiting and delayed intervention was carried out in 134 in men with tumors of T1b-T2b N0 MO, Gleason $\leq$7, PSA $\leq$ 15. The minimum F/U was 12 months (median 24). The distribution of PSA DT was: <2 yr 19 patients; 2-5 yr, 46; 5- 10 yr, 25; 10-20 yr, 11; 20-50 yr, 6; >50 yr, 27 ! The median DT, primarily of arithmetic interest, was 5.1 years and >33% had DT > 10 years. Other studies have shown a "median" PSA DT of 4 - 5 years.

And then ... with castration this slowly constructed tumor mass, in one decimating apoptotic collapse, comes crashing down in less than 21 days (2 or 3 months for medical castration)! In "Quantitation of Apoptotic Activity Following Castration in Human Prostatic Tissue in Vivo", The Prostate 54:212, 2003, Staak presents his evaluation of the apoptotic effect of castration on human PC cells transplanted under the renal capsules of mice. The baseline cell death rate was .026%. After castration the apoptosis quickly reached a maximal rate of 1.54% at 3 days, i.e. 60 X normal, and then gradually fell back to baseline by 21 days. By this time 87% of the cell mass had died! But unfortunately, some 13% of the cells remain viable ... the ones that require our continuing ministrations!

Bottom Line:The preclinical history of a prostate cancer is much longer than generally recognized.

DIAGNOSTICS Now the World Knows We Need a Better Mousetrap

Ever since the NEJM publication in July on "Effect of Verification Bias on Screening for Prostate Cancer by Measurement of Prostate-Specific Antigen", the news media has heralded the deficiency of the PSA test in prostate cancer detection. Seattle Times, July 30: "...PSA test - probably misses more than 80% of the cancers in men younger than 60 and almost two-thirds of the cancers in older men." [!] Unfortunately, until (and if) rapid, high-throughput throughput serum proteomics or other new approaches come to our rescue, we are left with rearranging the various permutations of the "PSA" molecule to gain greater test specificity.

A claim of greater test specificity was recently made by Mikolajczyk, Beckman Coulter, Inc., in his presentation at the Annual Meeting of the American Association for Cancer Research (published in Keio Journal of Medicine, 52(2):86-91, June 2003). His Beckman group "have developed highly specific and sensitive research immunoassays for BPSA [associated with BPH] and the different forms of proPSA.

A bit of background biology: In normal prostate glands proPSA is secreted into the glandular lumen where seven amino acids are cleaved to create active PSA, some of which gains access to the circulation where it is immediately complexed. In cancer, presumable because of disruption of the basement membrane, the proPSA enters the circulation directly and, by avoiding entering the lumen, escapes the enzymatic conversion into active PSA. The result is greater quantities of proPSA in the serum. The measurement of this proPSA is the basis of their test, which aims at better detection of the prostate cancer, present in 20 - 30% of men in the 2 - 4 ng/ml PSA range.

Results: When the proPSA test was set at a sensitivity of 90% and applied to men within the PSA range of 2.5-4 ng/ml "the specificity for the proPSA was 25% compared to 10% for %free PSA and complexed PSA (P=<0.001). And this increased specificity was maintained in the PSA range of 4 - 10 ng/ml.

Bottom Line: Any increase in the specificity of "PSA" testing will help to avoid negative biopsies.

DIAGNOSTICS Is Occult Cancer in Lymph Nodes the Achilles Heal of Successful treatment? A Few Cells May Cast a Long shadow.

[The September Commentary presented data indicating that extended lymphadenectomies find more metastatic spread in nodes than is found in more limited procedures; in the August Commentary the possibility that Combidex MR imaging may lower the threshold for detection

of nodal cancer to 5 mm masses. This article addresses the early application of sentinel lymph node mapping technique to this problem.]

For more than forty years clinical researchers of breast cancer have exhaustively studied the extent of axillary nodal metastases from the primary breast lesion, correlating their findings with the risk of relapse, and using the information as the basis of therapeutic decisions. They developed a method for identifying the sentinel axillary lymph node, the first node in the nodal chain. As collective experience matured, clinicians have become more confident that a pathologically negative sentinel node implies with >95% accuracy that neither macroscopic nor microscopic spread is present in the unresected remaining nodes. Although it is still controversial as to the significance of the extreme minimum of cells, i.e. those metastatic cells only identified by immunocytochemistry or seen only in the nodal sinuses, none the less, there is agreement that cell clusters as small as 2 mm adversely affect outcome. This observation has been found to apply to lung cancer, esophageal cancer and other cancers, and it would be surprising if prostate cancer was an exception.

The premises of the following discussion are: 1) in prostate cancer surgery if the node sampling is limited to only nodes in the obdurator and external iliac chains, a significant number of positive nodes will be missed; 2) sentinel lymph node (SLN) mapping with technetium-99m nanoparticles, although in an early stage of clinical usage, can identify the principle first node in the draining pathway and guide the surgeon's selection for excision to one or two significant nodes; 3) by limiting the pathologists' task to examining only these one or two nodes extensive thin sectioning and immunohistochemical analyses can be efficiently employed; and 4) accumulating experimental results will define which patients with apparently localized disease should be subjected to SLN studies.

Wawroschek and colleagues in Augsburg, Germany, (Eur Urol.2003 Feb;43(2) 132-6) found 26.8% nodal positivity in 194 patients with apparently clinically localized prostate cancer by doing an extended lymphadenectomy (LAD). After retrieving the SLN, which had been identified by Tc-99m, the various surgeons carried out modified or extended LADs. "At first all patients had a sampling of the sentinel lymph nodes followed in most cases by a modified or extended pelvic lymphadenectomy. Step sections, serial sections and immunohistochemistry (IHC) were analyzed in all SLN and so-called nonSLN of the first 100 patients. Later serial section and IHC of non-SLN nodes were left out." They concluded that by examination of just the obdurator nodes only 44.2% of the total positive nodes would have been found; the additional inclusion of nodes from around the external iliac vessel only improved the sensitivity to 65%.

Additional support for these conclusions comes from a study by Heidenreich, Philipps-University Marburg, Germany, (J Urol. 2002 Apr;167(4):1681-6) who based his rationale for extended LAD on the awareness that the prostate's principle lymphatic drainage is to the internal iliac and the presacral nodes. A total of 103 consecutive men with clinically localized prostate cancer received a radical prostatectomy and accompanying lymphadenectomy which included retrieval of nodes from the external and internal iliac, the obdurator, common iliac, and presacral lymphoid areas. A mean of 28 nodes were examined per patient and 26.2% were positive. For the 27 patients with positive nodes one, two and three nodes were positive in 15, 9, and 1 patients, respectively. They compared the surgical complications in their extended LAD series with 100 patients with RP and standard LAD. "There were no significant differences in regard to intraoperative or postoperative complications, lymphocele formation or blood loss between the two groups." A useful observation was that 95.8% of the patients with positive nodes had a PSA of >10.5 ng/ml and a Gleason score $\geq$ 7. Patients with less than

those values were therefore identified as a low risk group where the risk of nodal positivity was 2%. Epstein and Partin (CANCER, Sept. 1, 2002; p. 1016) reported in a study of 443 patients who underwent sextant biopy and RP with lymphadenopathy that the risk of nodal spread was 2.2% in instances wherein none of 6 biopsy cores had a major Gleason pattern 4 or only $\leq$ 3 showed any minor pattern 4.

Reports of sentinel node mapping are just emerging in the literature. The article by Wawroschek (Urol Int.2003;70(4)303-10) is a good primer. They performed SLN mapping in 350 patients. The procedure began the day before surgery with an ultrasound guided transrectal injection of 2-3 ml of 99mTc radiolabled particles dividing half the volume per lobe. Lymphoscintography was then performed. At the subsequent operation those nodes that were identified as SLN by means of intraoperative gamma probe detection and prior lymphoscintography were removed. After retrieval of the SLN, a modified or extended LAD was performed. Results: 335 of 350 patients showed at least 1 SLN; 24.7% had lymph nodes metastases, and there were 2 false negatives.

Bottom Line: Sentinel lymph node mapping is emerging as a technique to guide urologists in selecting which node (or nodes) to sample. Also, guidelines are developing to indicate which patients should undergo SLN testing.

PATHOLOGY Does 3 + 4 and 4 + 3 Always Equal 7?

Not quite - when the issue is the influence of Gleason scores on treatment outcome. In the prostate cancer world when adding Gleason grades to arrive at the Gleason score, it is more like 3 + 4 = 7- and 4 + 3 = 7+. Practitioners in prostate cancer know this. However, it was very clearly set out by a group at MCKCC in "The Prognostic Significance of Gleason Grade in Patients Treated with Permanent Prostate Brachytherapy" (Int J Rad Onc Biol Phys Vol.56, pp.749-754, 2003). They studied 1029 T1/T2 PC cases with Gleason sums 6, 7, 8 with a median F/U of 46 months. Failure was set (ASTRO definition) at the midpoint between the posttreatment nadir and the first of three consecutive PSA rises. Results at 7 years: biochemical freedom from relapse for GG 3 + 3 was 81.8%; for GG 3 + 4, 78.4%; for GG 4 + 3, 56%; and for GG 4 + 4, 50.7%. Similar findings for outcome post RP have been published. Herman, Kattan, Scardino, AM J Surg Pathol 2001, 25(5):657-660, reported the findings from their study of RP in 832 cases. Although the primary Gleason grade 4 lost independent significance when collated with the available information regarding tumor volume, surgical margins, and the status of seminal vesicles, extracapsular extension, and lymph nodes, none the less, the primary Gleason grade 4 remained significantly correlated with the other predictors of progression.

Bottom Line: When reported by excellent pathologists, major Gleason pattern 4 carries adverse prognostic significance.

UPCOMING LECTURE: . Michael Kattan of MSKCC, principle author of the "Kattan Nomograms", will give a lecture Thursday, October 16th, 7:30 AM in the Glaser Auditorium, Swedish Hospital, and then speak again at the GU Tumor Board, Friday, October 17, 12:15 PM