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DNA Ploidy: Early Malignant Lesions

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Abstract The nuclear DNA content of prostate cancer specimens, both needle biopsies and aspiration biopsy specimens as well as transurethral resection (TUR) chips and radical prostatectomy specimens, can now be reliably measured by standardized methods of flow and static image cytometry. For prostate carcinomas of every clinical stage (A1-D2), DNA diploid tumors have a better prognosis than tumors of a similar stage and grade which are non-diploid. Of particular importance to this symposium is the fact that DNA diploid stage D1 and D2 tumors treated early by androgen deprivation generally have a remarkably good prognosis. In contrast, those patients with DNA non-diploid tumors progress early despite androgen deprivation. Such a result suggests that DNA ploidy can be used to identify prostate cancers which are potentially sensitive to hormonal manipulation. Additional investigations from several groups indicate that early stage prostate malignant lesions, for example stages A1, A2, B1, and B2, are generally DNA diploid (about 75%). Swedish data suggest a steady progression of prostate cancer from early diploid to tetraploid, to non-tetraploid aneuploid, to multiple stemline aneuploid tumors with time and advancing stage. Taken together, these data suggest that the earliest detectable prostate carcinomas should be overwhelmingly DNA diploid. A large majority of these patients with early tumors should be candidates for "chemoprevention" by pharmacologic methods which reduce the effective androgen stimulation of prostate tumor cells.

Key Words: androgen ablation, DNA ploidy, early prostate carcinoma

DNA PLOIDY OF EARLY PROSTATE TUMORS

Many published studies have investigated DNA ploidy of low stage prostate cancers. Most of these studies have been done by flow cytometry using both fresh prostate cancer samples as well as nuclei extracted from formalin-fixed, paraffin-embedded archival samples. A number of studies, particularly from Europe, have used static image cytometry on sections from fixed tissue stained with the Feulgen technique. These studies have similar results. That is, cells of prostate cancers which are clinically or pathologically confined within the prostate capsule are generally DNA diploid (or normal).

This result has been found in a large series of Swedish patients from whom tumor cell samples were obtained using Franzen needle aspirates. In the representative study by Tribukait *et al.*, approximately 80% of early stage tumors were diploid [1]. In our own Mayo Clinic experience, more than two-thirds of pathologic stage B tumors treated by radical prostatectomy were DNA diploid [2]. Within this series, if one looked at the earliest stage tumors (so-called "B1 nodules"), over 75% were DNA diploid. This result contrasts distinctly with higher pathologic stage tumors. For example, in our Mayo Clinic series of pathologic stage C tumors, only 50% were diploid [3]. Forty percent of the tumors that were D1 at the time of surgery were diploid [4]. Thus, as prostate tumors become higher stage, they are more likely to have an abnormal ploidy pattern, either DNA tetraploid or non-tetraploid aneuploid.

Similar results have been reported from Sweden by Adolfsson and Tribukait [5]. In their series, patients were diagnosed with prostate cancer by aspiration biopsy. No active treatment was used until the tumor progressed clinically. During the period of observation, DNA ploidy determinations were made by aspiration of the tumor at 18 month intervals. These investigators found a steady conversion of diploid to non-diploid tumors over time, with kinetics which were very suggestive of radioactive decay [1]. They even suggested conversion of tumors from

diploid to tetraploid and from tetraploid to non-tetraploid aneuploid at a rate of 9% of tumors per year. In another series from Stanford, Dr. Jones and others reported that the small volume, stage B tumors studied were DNA diploid [6].

All of these investigations clearly indicate that most early stage prostate cancers, certainly more than 75%, will be DNA diploid. Since the earliest histologically detectable stage, *e.g.*, the 1 mm diameter microtumor, is currently not detectable in living patients, the probability of finding a diploid tumor phenotype might be much higher than 75%.

DNA PLOIDY AND RESPONSE TO ANDROGEN TREATMENT

If very small, subclinical prostate cancers are the general target for "chemoprevention" in men aged between 50 and 65, why is the DNA ploidy status of the tumor important? The most recent data suggest that the DNA ploidy of prostate cancer may be the best prognostic parameter available for predicting responsiveness to androgen deprivation. This effect can be seen in the very first studies of DNA ploidy and prostate cancer carried out in Oporto, Portugal, by Dr. Tavares and colleagues beginning 25 years ago [7,8]. Even with the relatively primitive cytometry techniques available at that time, it was evident that those patients with prostate tumors of relatively normal DNA content had a much better prognosis (in terms of cause-specific survival) than those whose prostate tumors had an elevated DNA content. This observation was based on a small clinical series in which patients with fairly advanced tumors were treated by hormonal manipulation.

Many additional studies from around the world have demonstrated similar results, *i.e.*, patients with normal DNA content (or DNA diploid) tumors have a much higher probability of a long term response to androgen ablation than patients with higher nuclear DNA content or non-diploid tumors. Typical examples can easily be identified in the Scandinavian literature in which DNA ploidy and response to androgen manipulation have been heavily investigated over the past 20 years. Representative examples might be the reports of Kjaer and Zetterberg and colleagues in which the contrasting responses to androgen manipulation between patients with diploid tumors and non-diploid tumors were particularly well demonstrated with long term followup [9,10]. Miller and colleagues from Adelaide, Australia, reached a similar conclusion [11]. In this retrospective study, patients who presented with osseous metastases were treated by bilateral orchiectomy. Many of the patients had transurethral resection (TUR) chips in the tumor archives which could be studied by Hedley technique flow cytometry for DNA ploidy. Those patients who died within one year after diagnosis had DNA aneuploid tumors. The vast majority of patients who survived more than 5 years after orchiectomy had primary prostate tumors which were DNA diploid. Thus, even for patients with widespread metastatic prostate cancer, the DNA ploidy of the primary tumor was strongly predictive of the response to androgen manipulation.

We have found similar results in our Mayo Clinic studies of patients treated by radical prostatectomy. A large number of patients were thought to have surgically treatable lesions (stage B, pT0–T2), but at the time of exploration were found to have a few metastatically involved pelvic lymph nodes. A radical prostatectomy was performed on those patients with so-called stage D1 disease. Many of these patients were treated by early androgen manipulation, either bilateral orchiectomy or diethylstilbestrol (DES) or both; however, another group of stage D1 patients was treated hormonally only when the tumor had progressed clinically. This series of patients has been reported in several publications focusing on ploidy associations in the past several years [4,12]. The important conclusion from our Mayo Clinic experience is that those patients with stage D1 DNA tetraploid or aneuploid tumors do not have a good prognosis whether they receive early endocrine treatment or not; almost all of these patients progressed and many died within 10 years after treatment. By contrast, about 40% of the patients had DNA diploid primary tumors. Even though the tumors were metastatic to the pelvic lymph nodes, these patients experienced a much better prognosis [4]. Most remarkably, those patients with stage D1 diploid disease treated by early endocrine manipu-

lation and radical prostatectomy have had particularly excellent long term responses, with virtually no progression within 10 to 20 years of clinical followup [12]!

Such results emphatically suggest that DNA diploidy measured by flow or static cytometry is probably the best marker available in 1992 for identifying patients who will respond to androgen ablation treatment. Conversely, those patients with DNA tetraploid and aneuploid tumors are less likely to be long term responders. In general, those patients with non-diploid tumors of every stage and grade seem fated to have a poorer prognosis at the present time.

Why is all of this important in the "chemoprevention" of prostate cancer? The overwhelming majority of low grade, low stage prostate cancers (even subclinical ones) should be DNA diploid; these tumors should be remarkably responsive to androgen deprivation maneuvers, as are the much more advanced metastatic diploid prostate carcinomas. As discussed in other sections of this seminar, there is every reason to believe that high serum testosterone levels are necessary for the initiation and promotion of prostate carcinoma. If almost all of these "initiated" subclinical tumors are DNA diploid, androgen manipulation should be a successful "chemoprevention strategy" for preventing or significantly delaying subsequent prostate carcinoma progression. One need only find effective pharmacologic agents which reduce androgen stimulation of subclinical diploid prostate tumor cells without significant adverse effects. The most likely agents, in addition to direct anti-androgens, may be 5α-reductase inhibitors such as Finasteride, which reduce intra-prostatic dihydrotestosterone levels.

In conclusion, from the point of view of the "ploidy cytometrist," early prostate carcinomas are excellent targets for "hormonal chemoprevention." These tumors should usually be DNA diploid; for this reason, androgen ablation therapy should work in a particularly effective and profound way. Indeed, medical or surgical castration would also probably be effective for such patients, as it is in stage D1 or D2 diploid disease; however, the side effects would be unacceptable in a "chemoprevention" study. Pharmacologic techniques which can significantly reduce androgen stimulation of the prostate should work just as well for "chemoprevention" of progression of DNA diploid prostate malignancies.

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