Chromosomal instability detected by interphase fluorescence in situ hybridization and its relation to p53 alteration in prostate carcinoma in Saudi patients

Jaudah A. Al-Maghrabi, MD, FRCPC.

ABSTRACT

Objectives: Chromosomal instability (CIN) is a feature of human neoplasm. The p53 mutation has been shown to be associated with CIN in many human dysplastic and neoplastic lesions. The objective of this study was to examine CIN and p53 mutations in prostate carcinoma (Pca) resected from Saudi patients.

Methods: Testing of p53 alteration using immunohistochemistry was performed on 28 archived prostatic carcinoma specimens containing Pca foci from Saudi patients seen at King Abdul-Äziz University Hospital, Jeddah, Kingdom of Saudi Arabia. Chromosomal instability was evaluated in the same tissues by interphase in situ hybridization (IFISH) using centromere probes for chromosome 7 and 8. Immunohistochemistry and IFISH were performed at Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada in 2001.

Results: The p53 immunoreactivity was found in 29% in Pca and 0% in benign epithelium. Interphase in situ hybridization revealed numerical chromosomal alterations in keeping with CIN in 63% of p53 positive and 20% p53 negative Pca. No evidence of CIN was seen in non-neoplastic epithelium.

Conclusion: We concluded that CIN as determined by IFISH is present in Pca from Saudi patients similarly to those reported in western countries. The p53 mutation occurs relatively infrequently in Pca and is associated with the presence of CIN at least in a subset of Pca.


Recently, we demonstrated that chromosomal instability can occur in the preneoplastic stage of prostate carcinoma (Pca) or what is known as prostate high grade intraepithelial neoplasia (HPIN) and that was an additional evidence in support of the concept that HPIN might be the earliest precursor of cancer.1-3 In those studies we demonstrated higher frequency of chromosomal instability (CIN) in Pca than in intraepithelial neoplasia (PIN). The reported frequency of mutation of the p53 tumor suppressor gene in Pca has varied widely, ranging from 3-72% in carcinomas of the prostate.1,4-10 In the literature there is controversy on the question of whether p53 alteration is an early or late genetic change.4,9-15 Striking heterogeneity of p53 mutation in prostate cancer has been reported16 and different mutated alleles were found among multiple tumor foci in single glands.16,17 The p53 has been found to be associated with genomic instability leading to chromosomal rearrangement, which in turn has been
Figure 1 - Foci of prostate cancer with p53 immunohistochemistry and interphase in situ hybridization. a) Hematoxylin and eosin sections showing foci of prostate cancer. b) p53 immunohistochemistry (DO7). Shows positive nuclear staining in invasive cancer. c) Interphase in situ hybridization on a focus of invasive prostate carcinoma using centromere probe for chromosome 7. Some cells show more than 2 red signals consistent with a gain of chromosome 7. d) Interphase in situ hybridization on a focus of invasive prostate carcinoma using centromere probe for chromosome 8. Some cells show more than 2 green signals consistent with a gain of chromosome 8.

demonstrated as a feature of many neoplastic and preneoplastic (dysplastic) human epithelia. The objectives of this project was: firstly to study the p53 mutation pattern and CIN in Pca of Saudi patients and secondly, to study the relation between p53 mutation and chromosomal instability in prostate epithelium.

Methods. Tissue samples were obtained from prostate carcinoma seen at King Abdul-Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia (KSA). A total of 28 cases were included in the study. Interphase in situ hybridization (IFISH) has been performed on 4-5 micron unstained tissue sections of the same blocks used for the p53 study, using adjacent hematoxylin and eosin (H & E) stained sections as guidance. Directly labeled VYSIS CEP probes for chromosomes 7 and 8 have been used. Paraffin pretreatment and in situ hybridization (FISH) procedure has been performed as it has been previously described. Dual-probe hybridization has been performed. For each probe 100 nuclei have been counted. All the H & E slides have been reviewed to confirm the diagnosis and to determine the adequacy of the specimen for FISH analysis. Only those with sufficient material were included in the study. Interphase FISH was performed successfully on 18 cases (8 of the p53 positive cases and 10 of the p53 negative cases). Slides were evaluated according to the accepted criteria. Briefly, only sections with hybridization in at least 80% of cells were evaluated. The number of signals per nucleus has been scored as (0, 1, 2, 3, 4, and >4) signal per nucleus. Nuclei from stromal element have not been enumerated. In situ hybridization by using a centromere probe for chromosome 4 was used as a negative control. Normal and hyperplastic glandular epithelium present in the biopsies was counted as internal control. Due to truncation of the nuclei, artifact loss of signals was expected; however very conservative criteria have been applied to detect any significant true numeric changes. The criteria to evaluate numeric chromosomal abnormality was as follows: 1. Chromosomal gains have been diagnosed when
more than 8% of the nuclei exhibit more than 2 signals. 2. Chromosomal losses have been diagnosed when more than 50% of the nuclei exhibit a reduction of signal number. 3. Tetraploidy has been assumed when all chromosomes investigated show signal gains up to 4. These cutoff values were adopted from the available literature.

Immunohistochemistry was performed on archival formalin fixed paraffin embedded sections (5 μm). Monoclonal antibody to p53 (DO7 clone; Novocastra Laboratories Ltd., Newcastle, England) was applied using avidin-biotin peroxidase complex (Elite kit; Vector Laboratories, Burligame, California). The positive control for p53 immunoreactivity (IR) consisted of formalin-fixed sections from bladder transitional cell carcinoma. Negative internal controls were stromal cells. Immunoreactivity was categorized semi-quantitatively from 0 to 4+ (0 = no IR, 1+ = 1-10%, 2+ = 11-40%, 3+ = 41-70%, 4+ = 71-100%). Staining was defined as positive whenever any specific nuclear brown staining is detected. Immunohistochemistry and IFISH were performed at Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada.

Results. We identified 28 transurethral prostatic resection specimens revealing Pca (Figure 1A). We have performed p53 analyses using immunohistochemistry (IHC) (DO7) on representative sections of these specimens. Eight cases (29%) stained positively for p53 in Pca foci (Figure 1B). Immunoreactivity in those positive cases was categorized semi-quantitatively as follows: 2 cases as 1+, 2 cases as 2+, and 4 cases as 3+. The normal, atrophic and hyperplastic tissue situated in the same sections showed negative staining in all the cases. The Gleason grade for p53 positive cases was 6 (1 cases) 7 (4 cases) and 8 (2 cases) and 9 (1 case). The Gleason grade for p53 negative cases was 6 (7 cases), 7 (10 cases) and 8 (2 case) and 9 (1 case). The volume of the tumor as evaluated by the percentage of the tumor in the tissue specimens for p53 positive cases was <10% (4 cases) and >10% (4 cases). For p53 negative cases the percentage of tumor was <10% (11 cases) and >10% (9 cases). When these results were compared to pathological findings, there was no statistically significant difference between the p53 positive and p53 negative cases regarding Gleason grade and volume of the tumor. Numerical chromosomal alterations in keeping with CIN were found in 5 cases (63%) of p53 positive and 2 cases (20%) of p53 negative Pca (Figures 1 C & D). Gain of chromosome 8 was the most frequent change in Pca followed by gain of chromosome 7.

Discussion. Carcinoma of the prostate occurs at a low frequency rates in KSA. The molecular and cytogenetic changes of Pca have not been studied in Saudi patients. Recently, using prostatic specimens resected from Canadian patients, we demonstrated that chromosomal instability can occur in the preneoplastic stage of Pca or what is known as prostate HPIN and that was an additional evidence in support of the concept that HPIN might be the earliest precursor of cancer. In agreement with other studies our result showed that p53 mutation occurs relatively infrequently in Pca (28%) compared to other human cancers like colon, esophagus and lung cancer. Our study did not show positive nuclear staining in the adjacent normal, hyperplastic or atrophic foci including those tissues adjacent or intermingled with cancer foci in any of the cases. Generally, a good correlation between p53 alteration detected by IHC and molecular studies has been noted in prostate cancer. Hall et al found complete agreement between IHC and TP53 Single Strand Conformation Polymorphism analysis. Wertz et al reported 85% overall agreement between the 2 methods while the concordance was 76.7% by Salem et al. Our study, as well as some other recent studies (both in-vitro and in-vivo) has demonstrated such correlation between loss or mutation of p53 and chromosomal instability. More recently, centrosome hyperamplification was found to be the major mechanism responsible for chromosomal instability in-vitro and in-vivo. Centrosome is the major microtubule-organizing center and required for spindle bipolarity, spindle microtubule assembly and balanced segregation of the chromosomes. A very strong correlation has been found between p53 loss or mutation and centrosome hyperamplification. Breast carcinoma and squamous cell carcinoma of the head and neck with either p53 deletion or mutation, show centrosome hyperamplification. Interphase FISH analysis for chromosomes 7 and 8 was performed in this study to assess CIN. We used these chromosomes to assess CIN as they are the most frequently affected chromosomes in prostate cancer pathogenesis. Although, CIN represents generalized changes in the cellular chromosomes, it is selective for certain chromosomes in carcinogenesis of different organs. Our finding revealed numeric chromosomal aberrations in 5/8 and 2/10 of p53 positive and p53 negatives. No CIN has been detected in the normal, hyperplastic, or atrophic epithelium and those areas showed no p53 alteration either. Recently, we demonstrated that p53 mutation may play a role in the progression of HPIN to invasive cancer and this could happen through induction of chromosomal instability. In this study, we applied IFISH on sections from the same blocks that have been used for p53 IHC and that enabled us to compare the findings of the 2 assays in the same foci of tissue.
Interphase FISH has higher sensitivity than other methods used for this purposes such as comparative genomic hybridization, which detects copy number changes if they are present in more than 50% of the cell population. Interphase FISH can identify CIN in a small subpopulations of interphase cells, allowing the detection of infrequent, possibly random changes before they lead to clonal expansion. Using IFISH on pretreatment and post anti-androgen therapy prostate cancer specimens, Karashima et al. found a remarkable reduction in the number of cells with extra copies of chromosome 7 and 8. Our IFISH results showed that gain of chromosome 8 is the most frequent finding in Pca. The c-Myc gene is located in the 8q arm and gain of chromosome 8 indicated an extra copy of that important oncogene. The role of c-Myc in the mechanism of CIN has been recently described. Extra copies of the c-Myc gene were identified in 52% of the HPIN and 44% of the carcinoma foci. Recently, we demonstrated that telomere erosion may be a consistent feature of Pca oncogenesis and may also be associated with the generation of chromosomal instability that characterizes this malignancy. Other possible mechanisms may involved in causation of CIN such as hypomethylation, activation of certain genes or inactivity of tumor suppressor genes.

We concluded that chromosomal instability as determined by IFISH is present in Pca from Saudi patients, similarly, to those reported in other countries. The p53 mutation occurs relatively infrequently in Pca and is associated with the presence of CIN at least in a subset of Pca. Although, there is a clear difference in the incidence of Pca between KSA and the western countries, this neoplasm seems to have some common features at the genomic level.

Acknowledgment. I would like to thank Dr. Jeremy Squire from Ontario Cancer Institute, Princess Margaret Hospital, 610 University Ave, Toronto, Ontario, Canada, for his help and support in the FISH performance.

References


Chromosomal instability in prostate carcinoma ... Al-Maghrabi


Chromosomal instability in prostate carcinoma ... Al-Maghrabi


