Genetic Instability in Urological Cancer

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Key Words protein expression; DNA mutation; microsatellite instability; Urological tumor.

Abbreviations: MMR: mismatch repair, MSI: microsatellite instability, HNPCC: Hereditary non-polyposis colonrectal carcinoma, LMP: low malignant potential, LOH: loss of heterozygosity

Defects of MMR genes have been identified in many kinds of tumors. Loss of MMR function has been linked to genetic instability especially microsatellite instability that results in high mutation rate. In this review, we will discuss the microsatellite instability observed in the urological tumors. We will also discuss defects in the DNA mismatch repair in these tumors and their correlation to the microsatellite instability, as well as the gene mutations due to the microsatellite instability in these tumors. From these discussion, we try to understand the mechanism of carcinogenesis in urological tumors from the aspect of genetic instability due to mismatch repair defects.

1. Microsatellite Instability

There are many repetitive sequences in eukaryotic genome. These sequences, called microsatellite, are in tandemly repeated mono-, di, tri, tetra, or pentanucleotide motifs. They are widely distributed on every chromosome in the human genome. Furthermore, the length, nucleotide composition of the repeat unit and in the number of repeat units can be quite different. Microsatellite sequences have been used as markers in the mapping process of human genomes and other mammals.

The development of a tumor from normal cell occurs in multiple steps, requiring several changes in genetic information. In normal cells, the spontaneous mutation rate is quite low. A general consideration argues that the large numbers of mutations frequently reported in human cancers cannot be accounted for by the rate of spontaneous mutation observed in normal human cells. Many evidences are consistent with this hypothesis. One of the evidences is the instability consisting of large repetitive units, that is, microsatellite sequences. Using polymerase chain reaction (PCR) and electrophoresis analysis, alterations in microsatellite can be detected. This alteration of microsatellite from same individual is called as microsatellite instability (MSI). Microsatellite sequences are highly unstable in a subset of tumor cells from cancer patients as compared to normal cells. This instability is characterized by increases or decreases in the number of repeats, which can change by one or more. A hereditary form of colon cancer, hereditary nonpolyposis colorectal carcinoma (HNPCC), has a genome-wide instability of repetitive DNA sequences[1]. Microsatellite instability was also found in some cases of sporadic colon cancer not related to HNPCC[2], lung[3], breast[4], stomach[5], pancreatic cancer[3]. Thus, the defective replication processes might yield a high mutation rate, especially in repetitive sequences, and allow accumulation of mutations in cancer related genes in cells which might lead to carcinogenesis and/or tumor progression[6].

2. Microsatellite Instability and Mismatch Repair

Instability of microsatellite sequences at multiple genetic loci may result in mismatch repair errors. Mismatch repair (MMR) is one of the systems that recognizes and repairs misincorporated nucleotides during DNA replication. In eukaryotes, the MSH gene products (MSH1-6)[7], and MLH1, PMS2, and PMS1 products[8,9] are involved in the beginning phase of the MMR, and PCNA, DNA polymerase, DNA ligase are involved in the later event of MMR. Among the six products of MSH genes, MSH2 forms heterodimers either with MSH6 or MSH3. The MSH2/MSH6 complex binds most base/base mismatches and small insertion/deletion loops (IDLs) while the MSH2/MSH3 complex preferentially binds to small and large IDLs[10]. The MLH1 binds more predominately to PMS2 than to PMS1, and MLH1/PMS2 heterodimer plays a major role in the

MMR [11]. Mutations of hMSH2, hMLH1, hMSH6, hPMS2, and hPMS1, have been found in most cases of the hereditary non-polyposis colorectal cancer (HNPCC), and a subset of sporadic colon tumors. The mutations in MSH2 and MLH1 account for the majority of HNPCC (92.7%), while mutations in MSH6 are uncommon (6.6%) and mutations in PMS2 and PMS1 are rare [12].

3. Oncogenes related with microsatellite instability

Microsatellite sequences are widely distributed in all genomic DNA. If the microsatellite repeat is located in the coding region of a gene, the changes in the length of the repeats can cause frameshift mutations in that gene. Genes known to contain short tracts of repeated nucleotides in their coding sequence are regarded as targets for mutations in these tumors. This change will either lead to a truncated protein product or nonfunctional protein. Frameshift mutation resulted from MSI has been reported in several cancer related genes, such as TGFβ RII, Bax, MSH3, and MSH6, E2F4, BRCA1, BRCA2, and PTEN. TGFβ RII is a protein involved in the TGFβ signaling pathway that regulates the cell growth and differentiation and is one of the most important mechanisms in the maintenance of epithelial homeostasis. Alterations leading to either the repression or enhancement of this pathway have been shown to affect cancer development. Bax is a proapoptotic gene that contains a tract of eight consecutive deoxyguanosines in its third coding exon. It is a member of the Bcl-2 family, regulates the apoptotic pathway that involves both Bcl-2 and p53. Bax mutations are found in hematological malignancies, colon cancer with microsatellite mutator phenotype, and some other cancers. Also when people looked for somatic mutations in a tract of eight deoxyguanosines (G)8 within third exon of Bax in transitional cell carcinoma of bladder, renal cell carcinomas cell lines and tumor specimens as well as prostate tumor specimens, no genetic alterations, at least in this specific region of Bax gene in urological cancers. It indicated that alterations of Bax, at least at investigated specific region, do not play considerable role in renal cell carcinoma, transitional cell carcinoma or prostate tumorigenesis[13, 14]

The insulin-like growth factor II receptor (IGFIIR) gene contains several repetitive sequences within its coding region, frameshift mutations in the poly(G)8 tract of IGFIIR has been found in 25% of gastric cancers, and 16% of colorectal cancers with MSI, which suggested that the IGF-IIR be implicated in the carcinogenesis of stomach, and colorectum cancer[15]. Mutations in IGFRII were more commonly found in those EC patients with lymph node metastases than primary tumors. This suggests that IGFRII may play a role in tumor progression in these patients[16]

E-cadherin is a cell adhesion molecule, loss of this gene is related to the less adhesiveness of cancer cells. It is considered to be involved in the process of tumor metastasis. In bladder cancer, the microsatellite analysis showed the presence of genomic instability in proximity of the E-cadherin gene. In one study there were nine out of 30 (30%) specimens presented molecular alterations in at least one out of 2 loci (D16S260 and D16S301) analyzed. The comparison between microsatellite mutations and clinical-histopathological parameters revealed a higher number of alterations in the invasive tumors than the superficial one ($p=0.014$). On the other hand, there were no statistical differences regarding the correlation with pathological grade[17]. In addition, an in-frame deletion of CAG repeats encoding 6 of 24 glutamine residues of the androgen receptor (AR) has been reported in a human prostate cancer[18].

4. Microsatellite instability in urological tumors

In Renal cell carcinoma (RCC), MSI may be an early molecular event associated with the pathogenesis of RCC. In one paper, loss of heterozygosity (LOH) and microsatellite instability (MI) in human renal cell carcinoma (RCC) were investigated in 22 RCCs. Out of 22 RCCs, four (18%) MI and 4 (18%) LOH were observed at the 3p21.1-p14.2 and 17q21, indicating the presence of genomic instability and the loss of tumor suppressor genes at these loci[19]

Wilms' tumor is the most common renal malignancy of childhood. Loss of heterozygosity (LOH) at 16q is seen in about 17% of cases and has been associated with a poor prognosis, additionally, MSI was present in a subset of tumor specimens suggesting that genomic instability exists in Wilms' tumor[20]

In transitional cell carcinomas of the bladder, somatic instability at microsatellite repeats was detected. Instabilities were apparent as changes in (GT)n repeat lengths on human chromosome 9 and in a (CAG)n repeat in the androgen receptor gene on the X chromosome. The finding that low stage tumors already had MSI (Ta-T1), suggesting that these alterations can occur early in bladder tumorigenesis[21]. The frequency of MSI is sometime higher in the invasive and undifferentiated tumors than that in superficial and differentiated forms [22]. Loss of MLH1 or MSH2 protein in bladder cancer has also been detected by the quantitative immunohisto-

chemical (IHC) image analysis. PCR-based allelotype analysis revealed complete loss of heterozygosity in three dinucleotide repeats lying within, or in close proximity to, hMLH1 and hMSH2 was rare for MLH1; and MSH2, however allelic imbalance was detected in hMLH1 (11/57) and hMS2 (10/55). These alterations in structure and expression of DNA MMR genes suggest that they may be involved in the tumorigenesis and/or progression of bladder cancer[23].

In prostate cancer, microsatellite instability has been observed. However, the frequency of microsatellite instability in advanced prostate cancers was not statistically different with that of incidentally discovered prostate cancer at transurethral prostatectomy (TURP) for benign disease. These data suggest that genetic instability is an early event in prostate carcinogenesis, and does not appear to influence prognosis [24]. Some study suggested that the microsatellite instability of dinucleotide tandem repeat sequences is much higher than trinucleotide, tetranucleotide and pentanucleotide repeat sequences in prostate cancer. The MSI with different lengths of nucleotide repeat sequences did not correlate with the stage and grades of prostate cancer[25]. In another study, paired microdissected samples from both untreated, locally advanced primary tumors and recurrences after conventional androgen-deprivation therapy (ADT) were analyzed retrospectively for microsatellite instability (MSI) and loss of heterozygosity (LOH) at nine loci on chromosomes 8, 18, and X by PCR. In parallel, prostatic carcinomas treated by radical prostatectomy and corresponding lymph-node metastases were analyzed in the same way. Although MSI can be found in advanced prostatic carcinomas, there was no statistically significance difference. Thus, MSI apparently does not play a major role in the progression of prostate cancer regarding androgen-independent growth or lymphogenous spread [26]. The incidence of prostate cancer is higher in North America than that in Asia. One study showed that the frequency of this mutator phenotype is much higher in the United States than Japan, reflecting racial differences in the molecular tumorigenesis of this malignancy [27].

In prostate cancer, mutations of MLH1, MSH2 have been found in the prostate cancer cell lines [28]. The prostate cancer cell line LNCaP did not express hMSH2 and was found to have a homozygous deletion of hMSH2 exons 9 to 16, which resulted in truncation of the protein. Another cell line, DU145, doesn't express MLH1 and PMS2. A mutation has been found in the splice acceptor between exon 1 and 2 of the MLH1 gene in DU145 cells line. This mutation causes an alternate splicing between exon 1 and exon 2 that resulted in frameshift mutation and caused an early truncation of MLH1 protein[28]. In primary prostate tumor, decreased or loss of MMR protein has been detected. This change can also be detected in the prostate intraepithelial neoplasia, indicating that the loss of MMR protein is an early event in the tumorigenesis of prostate cancer.

Carcinogenesis is a complex multistep process involving various genetic and epigenetic events in proto-oncogenes and tumor suppressor genes. PCR-based analysis for MI has frequently demonstrated alterations in microstatellite in the urological and gyneocological cancer. The MSI is associated with the defects of mismatch repair protein. The cancer-related gene containing microsatellite are the targets for the mutations due to MSI. These genetic changes might be served as a marker for clinical diagnosis, clinical treatment, and prognosis of the urological cancer.

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