

## INITIATION AND PROGRESSION OF SUPERFICIAL BLADDER CANCER: CAN GENES PROVIDE THE KEY?

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It has been estimated that approximately 200,000-250,000 new cases of bladder cancer are diagnosed worldwide every year, with approximately 120,000 cancer deaths. In Saudi Arabia, bladder cancer represents 3.6% of all newly diagnosed cancer, and is ranked as the 7th most frequent cancer among men and 18th among women.<sup>1</sup> Among the various histologic subtypes, papillary transitional cell carcinoma (TCC) is by far the most common. Clinical outcome in patients with bladder cancer is determined by a variety of factors such as size, multicentricity, histologic grade and stage. Several staging systems based on progressive depth of invasion of the bladder wall have been proposed. The TNM staging system categorizes tumors into noninvasive (Ta, TIS), invasive up to lamina propria (T1), superficial muscle invasive (T2), and deep muscle invasive (T3). The staging systems have been used to assign treatment and to assess prognosis.<sup>2,3</sup>

Transitional cell carcinomas which are limited to mucosa (Ta) and lamina propria (T1) are known to have a good prognosis and are usually termed as superficial carcinoma. This includes papillary transitional cell carcinoma and flat intramucosal carcinomas (TIS). The latter appears to be biologically different, since it has a much higher propensity for progression and will, therefore, be discussed separately. Superficial papillary carcinomas may be treated by endoscopic removal, however, 50% to 70% of these tumors may recur and 15% to 25% may progress to a higher stage tumor.<sup>2</sup> Unfortunately, no reliable criteria for predicting the risk of progression in these tumors have been identified. However, a critical review of superficial bladder cancer has revealed that this may represent a heterogenous group of tumors, although it is not certain whether or not these tumor groups signify any distinct prognostic categories. Thus, while some studies have demonstrated no significant prognostic differences between various tumors included in superficial bladder cancers, others have found a significantly higher risk of subsequent tumor progression in T1 as compared to Ta tumors. It has also been suggested that further stratification of T1 tumors based on the extent of lamina propria invasion may be justified. Thus, there may be prognostic distinctions among tumors that infiltrate the lamina propria only microscopically (T1a); those that invade up to the muscularis mucosae (T1b); and those that penetrate the muscularis mucosae to involve the deeper portion of lamina

propria (T1c).<sup>1</sup> However, these distinctions, although potentially useful, may be difficult to apply in practice. This is because the interpretation of the endoscopic bladder biopsies for precise pathologic determination of the extent of invasion may be hampered by a multitude of factors, including crushing artifact, cautery effect, tangential cutting and sampling errors. Even when the section quality is good, pathologic diagnosis of stromal invasion may not be reliable. Several studies have shown a poor reproducibility among pathologists in interpretation of the extent of invasion in superficial carcinomas diagnosed on endoscopic biopsies from the urinary bladder.<sup>4</sup>

In addition to the extent of invasion, the prognosis in superficial carcinoma may also depend on the histologic grade. Studies have shown that the risk of progression of superficial bladder cancer may vary considerably with the histologic grade. Chen et al. showed that in Ta tumors, progression was greater in grade 3 tumors (28.5%) than in grade 2 (9.0%) and grade 1 (2.1%).<sup>5</sup> These results emphasize the importance of histologic grade in predicting the clinical course of superficial bladder cancer. However, since the majority of superficial carcinomas belong to histologic grades 1 and 2, there is a clear need for recognition and identification of additional prognostic markers in order to predict which superficial cancer will recur or progress and which tumor will metastasize.

In recent years, numerous studies on the genetic aberrations in bladder cancer have been published from laboratories around the world. Carcinomas of all grades and stages have been studied, and several studies have provided insights into the initiation and progression of bladder cancer. Most recently, the research has focused on combining pathologic variables with molecular markers in order to develop a comprehensive profile of the tumors with respect to their clinical outcome and projected response to therapy. These investigations have provided a wealth of knowledge to enhance our understanding of the genetic basis of carcinogenesis and genetic events that determine the clinical behavior and patient outcome (molecular staging).

In normal biological condition, DNA is very stable and well conserved, and any errors in the base sequence are immediately corrected. The most important protein involved in the conservation of the integrity of DNA is p53. The gene for p53 is located on chromosome 17p13.1



FIGURE 1. A diagrammatic representation of important proteins involved in cell cycle control.

and encodes a 53kD nuclear phosphoprotein with DNA binding properties. It is involved in several different mechanisms, such as transcription, DNA synthesis, repair and programmed cell death. Changes affecting p53 protein may lead to accumulation of mutations in other genes. However, abnormalities of p53 gene are usually unrelated to the important early steps in the development of cancer, although they may play a crucial role in its further progression. P16 is another protein which plays an important function in controlling various steps in the cell cycle. This protein binds to cyclin-dependent kinase (Cdk2), and inhibits the phosphorylation of retinoblastoma protein, thereby inhibiting the cell cycle progression. The gene for p16 is located on chromosome 9p21 and is

regarded as a candidate tumor suppression gene, as the chromosomal region it maps is frequently altered in cancer.

The retinoblastoma susceptibility gene (Rb gene) encodes a nuclear phosphoprotein and maps on chromosome 13p14. Phosphorylation of Rb gene protein releases transcription factors which propel the cell into the next phase of cell cycle, namely S-phase (Figure 1). Loss of cell cycle control appears to be an important early step in the development of carcinogenesis and progression of various concerns. Several studies on clinical material have shown that reduced expression of Rb gene is associated with invasive properties and a high tumor grade.<sup>6,7</sup>

The development of new techniques in molecular genetics and cytogenetics of tumor cells has expanded the

search for useful prognostic factors in bladder cancer. In the early 1980s, cytogenetic analysis was used to identify numeric aberrations of various chromosomes, as well as interstitial deletions and loss of genetic material at specific locations. Later, several molecular techniques became available, such as fluorescent in-situ hybridization (FISH), restriction fragment-length polymorphism, polymerase chain restriction fragment analysis, comparative genomic hybridization (CGH) and microsatellite analysis. These techniques have provided a wealth of information on the processes involved in initiation and progression of bladder cancer.

Noninvasive (Ta) and early invasive (T1) superficial bladder cancers present the first steps of tumor development. Although by definition the only difference between Ta and T1 tumors is the presence or absence of invasion, there may be other differences which are not apparent on histologic evaluation alone. Recently, significant genetic differences have been found between Ta and T1 superficial bladder cancer. Simon et al., using CGH, showed that low-grade noninvasive papillary neoplasms (Ta) are characterized by paucity of gross genomic aberrations, except for the striking predominance of chromosome 9 losses.<sup>8</sup> These authors also showed that there is clearly a higher number of CGH alterations in T1 than in Ta tumors. Most of all, a much higher degree of genetic instability is suggested in T1 than in Ta tumors. This fits well with the considerably higher rate of aneuploidy of various chromosomes seen in T1 tumors.

Increasingly, there is a panel of individual genetic alterations that is recurrently being found in T1 carcinomas, including 1q+, 2q-, 8p-, 8q+, 10q-, 11p-, 11q- and 17p-. It is likely that some of these loci contain genes whose malfunction contributes to bladder cancer development and progression. However, it is not clear which of these genes are responsible for progression and aggressive behavior of the tumor and which ones are merely epiphenomena. In another study using fluorescent in-situ hybridization (FISH) technique, Pycha et al. evaluated 50 cases of Ta and T1 or higher stage carcinomas of the urinary bladder for the presence of numerical abnormalities in chromosomes 7, 9 and 17, along with ploidy status and Ki-67 and p53 immunoreactivity.<sup>9</sup> Of these patients, 29 had a primary Ta tumor while 21 cases with T1 or greater disease formed the controlled group. The data were compared to ploidy status and Ki-67 immune reactivity. All Ta tumors without recurrence were diploid or haploid, and revealed monosomy of chromosome 9 as the only numerical chromosomal abnormality. P53 immunohistochemical staining was also negative in all these cases, while there was a low percentage of Ki-67 staining. The tumors in patients with recurrence and progressive disease had a high incidence of trisomies 7 and 17, aneuploid DNA status, and high positivity for p53 and Ki-67. The authors concluded that aggressive types of superficial carcinomas may be identified on the basis of FISH findings. Several other

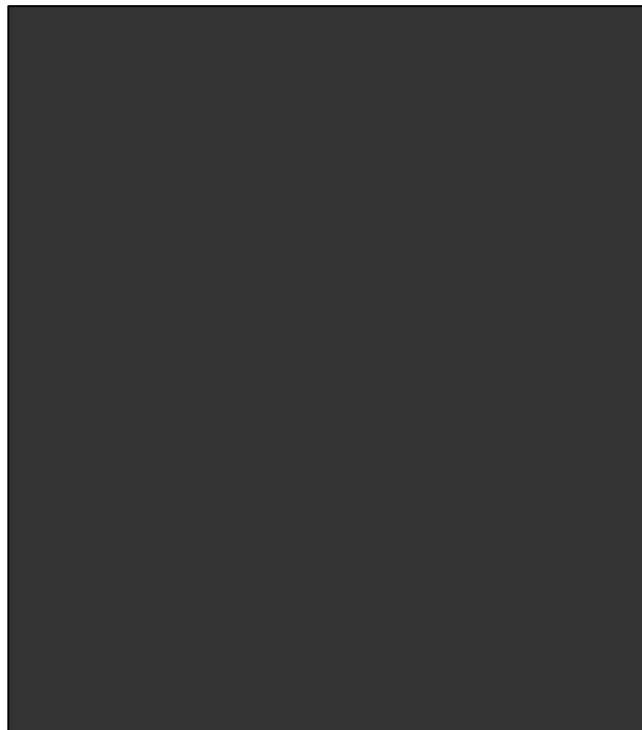


FIGURE 2. Idiogram of chromosome 9 featuring areas frequently involved in initiation of transitional cell carcinoma.

studies have provided evidence for numerical aberration of chromosomes 1, 4, 5, 7, 9, 11, 17 and 18 in both superficial and muscle-invasive bladder carcinoma.<sup>9,10</sup>

In addition to p53, pRb, which is the product of retinoblastoma gene (13q14) and is an important protein controlling the cell cycle, may also play an important role in determining the risk of progression and lengths of survival in transitional cell carcinoma. Cordon-Cardo et al. studied 59 patients with superficial TCC to assess the cooperative effect of alterations of p53 and pRb on progression and survival.<sup>11</sup> Patients who were p53-positive or had undetectable levels of pRb had more frequent disease progression and significantly decreased survival rate. If both markers were altered, the progression rate was much higher and survival was decreased further. These findings reveal that alterations of p53 and pRb have a cooperative negative effect on prognosis.

A consistent and perhaps the earliest genetic abnormality in transitional carcinoma involves chromosome 9.<sup>12,13</sup> Chromosome 9 alterations occur in bladder carcinogenesis and are partly independent of stage and grade. Approximately half of the bladder cancers have monosomy of chromosome 9, with deletion in the remaining chromosome. The deleted area involves 9p21-23 and 9q32-33 (Figure 2). In early disease, chromosome 9 deletion may be the only genetic abnormality, therefore suggesting a role in initiation of the urothelial cancer. It is a widely held view that the deletions in chromosome 9 are associated with loss of tumor suppressive genes, however,

none of these genes has yet been identified. A possible candidate at p21 may include alpha interferon gene and CDK (p16). Cytogenetic and molecular studies have revealed that monosomy or partial deletion of chromosome 9 is often the only chromosomal abnormality in early bladder cancer. Carcinomas with chromosome 9 aberrations as the sole abnormality are genetically stable and do not show progression. However, addition of other genetic abnormalities, such as p53 defect, trisomy 7 and LOH at 11p, among others, may indicate potential for progression.

Bladder cancers, especially papillary transitional cell carcinomas, are frequently multifocal, with several tumors arising from the urothelium at different times and at different sites. Clinically, metachronous multifocality is a major problem after endoscopic treatment of superficial urothelial cancers. Traditionally, the multifocal nature of urothelial cancer has been explained by the concept of "field cancerization," in which the entire urothelium is exposed to common carcinogenic insults resulting in multifocal urothelial tumors, presumably arising from independent clones of transformed transitional cells. However, detailed clinical observations have suggested that such multifocal tumors develop by seeding of viable cancer cells or by intraepithelial spread. This hypothesis is supported by several recent molecular genetic studies of multifocal urothelial carcinomas. For example, genetic analysis of multiple tumors from female patients showed that the same X chromosome was inactivated in tumors from different sites, and when 9q was lost, the same allele was deleted. In another study, Takahashi et al. studied 87 metachronous and/or synchronous urothelial cancers, and in 80% of the evaluable cases, the multifocal tumors were considered to be derived from a single progenitor cell.<sup>14</sup> The authors suggested that most multifocal superficial recurrent urothelial cancers are derived from a common clonal origin and are genetically stable with specific microsatellite alterations which may be used as a genetic marker for early detection of recurrence in the follow-up of urothelial cancer patients. Furthermore, most low-grade superficial papillary urothelial tumors seem to be genetically stable, although subsequent genetic divergence may occur in a subset of these tumors. Genetic divergence and heterotopic spread generally occur only after chromosome 9q and 9p alterations, which may occur long before the clinical manifestation of multifocal tumors.

Nonpapillary flat urothelial carcinoma in situ (TIS) is a full thickness proliferation of malignant urothelial cells confined to the epithelium.<sup>1,2</sup> The tumor cells lack cohesiveness and often exfoliate. TIS cannot be reliably or consistently recognized on cystoscopic evaluation. Most commonly, TIS is found in the vicinity of papillary tumors, although it may also be encountered in cystoscopically normal mucosa away from a visible tumor. On occasion, TIS may involve the urothelium in the absence of any accompanying papillary tumors. The involvement of urothelium in these cases may be quite extensive. The risk

of progression into invasive carcinoma is much higher in CIS than in papillary carcinoma (Ta).

Comparative studies on the molecular genetics of TIS and Ta urothelial carcinomas have revealed that these tumors are probably derived from distinctly different genetic pathways.<sup>7,15</sup> Whereas the earliest genetic aberration in papillary TCC may involve chromosome 9 deletions, TIS is characterized by abnormalities of the p53 genes. Loss of p53 function deprives the cell of an important surveillance mechanism necessary for maintaining the fidelity of the genetic material. This leads to accumulation of a variety of genetic aberrations which are responsible for the typical phenotype of CIS characterized by large hyperchromic and irregular nuclei. Progression of CIS may involve deletions of 9q and 9p and several additional genetic abnormalities, such as 3p, -6q, 2qp, -4p, 8p and 11p.

Although significant strides have been made in recent years in understanding the genetics of initiation and progression of bladder cancer, much still needs to be learned. It is hoped that as more and more cases are studied by ever advancing molecular genetic techniques, our understanding of the genetic events involved in bladder cancer will be considerably enhanced. This in turn will help in planning therapeutic modalities for these tumors which are more precise and specific for the patient.

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