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Chromosome Changes in Early Bladder Neoplasms

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Abstract There are few cytogenetic studies of early (non-invasive) bladder cancer, particularly *in situ* carcinoma, due to technical difficulties in examining such lesions. The best approach is to extrapolate from chromosomal changes in more advanced cancers. Although no specific chromosomal changes have been established in either early or fully developed bladder cancers, certain recurrent anomalies have been encountered. Anomalies such as +1, +7, –9, 5q– or i(5p), 11p– and –Y appear to constitute part of the multistep carcinogenetic process by which clinically and pathologically recognizable bladder cancers develop. Since loss of part or all of chromosome 9 (–9) is a common and early cytogenetic event in bladder cancer, the detection of –9 in bladder washings or urine by fluorescence *in situ* hybridization (FISH) may be a promising test for early or recurrent bladder cancer. Although less frequent than –9, trisomy 7 (+7) is common enough to serve a similar purpose. In contrast, loss of the Y chromosome may indicate an advanced stage of bladder cancer. Thus, FISH studies utilizing probes for chromosomes 7, 9, and Y should yield cogent information to identify early bladder cancer. Cytogenetic (including FISH) studies combined with certain molecular approaches (*e.g.*, p53 mutations detected immunochemically) may not only serve to differentiate early cancer from benign tumors or conditions, but may also help establish cancer stage. This would supply data of considerable usefulness to the clinician and pathologist.

Key words: bladder cancer, cytogenetics, FISH, molecular studies

There are several types of chromosomal (cytogenetic, karyotypic) changes in human neoplasms. Translocations as single cytogenetic events, resulting in the genesis of abnormal (chimeric) genes, are common in leukemias and in a significant number of soft tissue tumors, including sarcomas [1]. An even more common chromosomal anomaly, deletions, is most often associated with other cytogenetic changes, again usually deletions. In a significant number of situations, these deletions result in loss of chromosomal material thought to contain genes responsible for tumor suppression. More importantly, these changes are often seen in common tumors such as the (adeno)carcinomas of the lung, breast, colon, ovary and other tissues. Current thinking maintains that the acquisition of full malignancy in these cancers is associated with a multistep process of carcinogenesis. These steps may be detected cytogenetically; however, some can only be established molecularly. Cancer of the bladder appears to fall within the scheme just described, *i.e.*, the full development of the malignant state requires an orchestrated sequence of genetic changes.

The scheme presented in Figure 1 is in many ways hypothetical, but it does indicate the complexity of the genetic changes associated with the development of bladder cancer. Undoubtedly, this scheme will have to be modified as new cytogenetic and molecular data become available.

Cytogenetic studies on early bladder cancer, including superficial cancer and carcinoma *in situ*, are scarce [1]. However, the concept of the multistep process of carcinogenesis implies that most of the genetic changes shown in Figure 1 would have occurred once a tumor becomes clinically or pathologically manifest. It is clear that early bladder cancer can be expected to have at least a portion of the chromosomal and molecular changes seen in more advanced tumors. If this is the case, then examination of cells from bladder washings (and possibly from urine) for the most common chromosome changes could prove to be of value in the detection and follow-up of early bladder cancer. However, a major shortcoming in cytogenetically examining such cells is that they are not usually dividing and grow very poorly *in vitro*. The

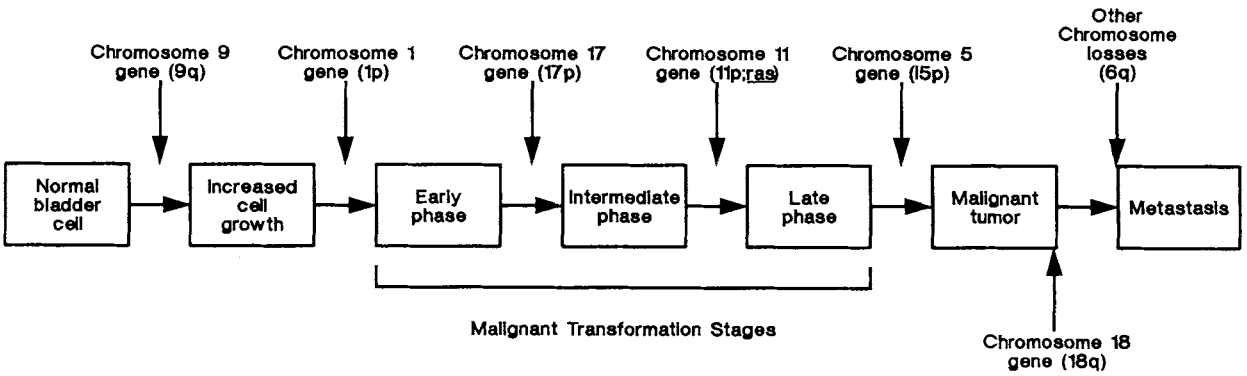

Fig. 1. Suggested pathway of the development of bladder cancer through a series of genetic alterations involving tumor suppressor genes (particularly in chromosomes 9, 17, 11 and 5) and oncogenes (*e.g.*, *ras* on chromosome 11). The accumulation of these changes gives rise to increasingly large tumors that eventually become malignant. With each progressive involvement of a gene or change, the affected cell becomes less and less responsive to normal control mechanisms, ultimately resulting in a tumor capable of invasion or metastasis. The chromosomes shown to be involved are based on our present knowledge, and this scheme or parts of it may have to be modified as more cogent information is obtained on events in bladder cancer. In addition, the scheme shown may apply to only a subtype of bladder cancer. In addition to deletions shown in this figure, loss of heterozygosity in bladder cancer has been described for 2q, 3p, 8p, 10q and 16q. Numerical changes may affect chromosomes 1, 7, 9, 11 and 20.

introduction of fluorescence *in situ* hybridization (FISH) techniques has made the detection of chromosomal changes, particularly numerical changes in interphase (non-dividing) cells, possible [1,2]. Since the karyotypic changes –9, +7, and –10 and loss of the Y are common anomalies in bladder cancer [1,3,4], FISH performed on exfoliative cells in bladder washings may be a sensitive and accurate approach to establishing the existence or recurrence of early bladder cancer when other approaches are equivocal or negative. Only 25–50 cell nuclei are required for such a FISH study and the results can be obtained within 1–2 days. In carcinoma *in situ*, the presence of only cytogenetically normal cells using several chromosome-specific probes (chromosomes 1, 7, 9, 10 and X and Y) is informative, since a normal karyotype suggests a lower potential for recurrence [5].

Total (–9) or partial (9q–) loss of chromosome 9 is a common cytogenetic event in bladder cancer, occurring in more than 50% of cancers at all stages and grades [6]. Loss of function of a gene(s) on chromosome 9 may be a key event in the development of the majority of bladder cancers; band 9q34 may be a locus for a tumor suppressor gene.

Table I. Common Chromosome Changes in Bladder Cancer

–9 or 9q–
+7
structural changes of chromosome 1
structural changes of chromosome 5
structural changes of chromosome 11
–Y

Loss of the Y chromosome (–Y) in bladder cancers of males is apparently unrelated to age, carries with it a poor prognosis (Fig. 2), and is usually indicative of an advanced stage [1,7]. In most cases the loss of the Y is part of a complex tumor karyotype; however, tumors with –Y as the sole anomaly have also been described [5]. Loss of the Y is a common and normal phenomenon in the bone marrow cells of elderly males, although no such loss was seen in normal urothelial cells. Thus, it is possible that loss of the Y in bladder cancer, particularly in early stages, may have a unique significance.

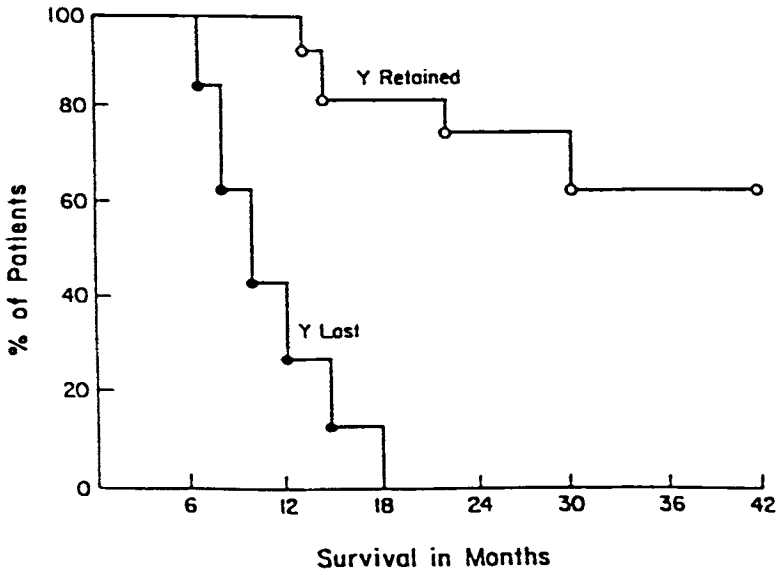

Fig. 2. Cumulative survival of male patients with bladder tumors with and without the Y chromosome. The loss of the Y was usually part of a complex tumor karyotype and did not appear to be age-related.

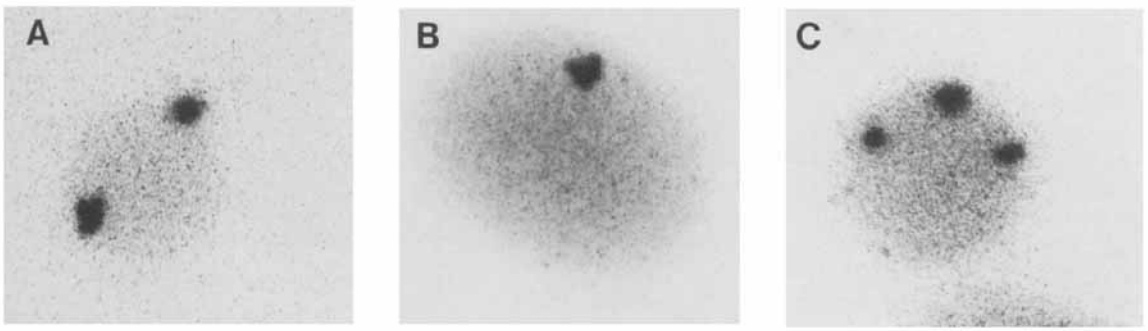

Fig. 3. Fluorescence *in situ* hybridization (FISH), shown in black-and-white, of bladder cells using centromeric alphoid probes [1] specific for the chromosomes indicated. Shown in A are the normal signals (2) for chromosome 9 in normal urothelial cells. In B, the same probe was used in a bladder cancer cell and demonstrates the absence of one copy of chromosome 9 as evidenced by a single signal. In C, a probe for chromosome 7 was used and the three signals indicate trisomy 7 (+7) in this bladder cancer.

The recognition of structural chromosome changes in interphase nuclei with FISH is, at present, difficult. Establishment of translocations by chromosome painting techniques are being developed in a number of laboratories. Their utilization in the detection of structural changes will be a major advance in the diagnosis of early bladder cancer.

Examination of Figure 1 offers hope in the diagnosis of early bladder cancer. The genetic events involved in the early steps of bladder cancer development may manifest themselves through specific changes, *e.g.*, in the urine or blood. The recognition of the cytogenetic changes would alert us to the development of a tumor and allow its early treatment. Similar recognition of subsequent genetic events associated with bladder cancer progression, particularly those occurring before invasiveness and metastases, may offer the clinician the opportunity to

cure the disease. Obviously, what is lacking at present is the ability to recognize these genetic changes, particularly the early ones.

Presently available molecular approaches may also prove to be of value in the early diagnosis of bladder cancer and in appraising the disease [8–10]. Changes (cytogenetic and/or molecular) on the short arm of chromosome 17 may lead to mutations and/or loss of heterozygosity (LOH) of tumor suppressor genes, particularly gene p53 on 17p (which can be detected immunochemically in affected cells). Mutations of p53 may be seen in all stages of bladder cancer, whereas LOH on 17p is present only in tumors of advanced stages. LOH of other genes such as H-*ras* [6], Rb [11], and *erb*B2 [12] may also indicate advanced stages of bladder cancer.

One study [9] showed that tyrosine-specific protein kinase activity of $pp60^{c\text{-}src}$, the protein product of the cellular proto-oncogene *src* located at chromosomal bands 20q11.2–q13, is elevated in a subset of human bladder carcinomas as compared with normal bladder mucosa. Increased protein kinase activity was observed mainly in low-grade bladder lesions and was associated, at least in part, with elevated levels of $pp60^{c\text{-}src}$ expression. Low $pp60^{c\text{-}src}$ kinase activity was found in cell lines originating from grade III bladder cancer, whereas increased activity was found in cell lines originating from grade I and II tumors. The authors suggest that the *src* proto-oncogene in urothelial cells may be associated with differentiation events [9].

When the cytogenetic (FISH) and molecular data are combined, they may supply information of crucial value to the pathologist and urologist in the diagnosis and care of patients with bladder tumors.

For more references, consult [1].

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