Urol Res (2001) 29: 377–387
DOI 10.1007/s002400100216

ORIGINAL PAPER

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The genetic alterations in the oncogenic pathway of transitional cell carcinoma of the bladder and its prognostic value

Received: 26 January 2001 / Accepted: 7 August 2001 / Published online: 19 October 2001

Abstract This review focuses on the main oncogenes studied in transitional cell carcinoma (TCC) in order to describe their mechanisms of action and investigate their possible prognostic value. Each oncogene family is reported following the order through which the proliferative signal is transduced from the extracellular space via a growth factor to the nucleus where transcription factors are switched on. Oncogenic activation at any level of the pathway will cause an increased transcription of genes enhancing the cell cycle, and proliferation will therefore be amplified. The main molecular or immunohistochemical studies from the literature on the aberrant expression of these genes are examined and compared with the aid of tables. Conclusions suggest that, although some may initially appear promising, no oncogene, has thus far been found to have a definite prognostic value superior to conventional grading and staging.

Keywords Transitional cell carcinoma · Oncogenes · Growth factor · Growth factor receptor · RAS · SRC · Transcription factor

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Introduction

Cancer is a disease of the genome. Early stages in cell transformation involve mutations of special genes directly or indirectly controlling cell proliferation and differentiation. When a crucial number of these events is reached, the malignant process is triggered and the cell undergoes phenotype changes involving loss of differentiation and uncontrolled proliferation.

Molecular biology has, in recent years, enabled the identification of some of these genomic "hot spots" where mutations seem to occur frequently in malignancies and may therefore account for carcinogenesis. The term mutation in this context indicates the entire spectrum of possible genetic alterations, including gene point mutations, deletions, or amplifications. These special genes can be divided in two categories: proto-oncogenes and tumor suppressor genes.

Proto-oncogenes are normal-cell genes coding for nuclear and/or cytoplasmic proteins and acting as promoters of cell proliferation. A proto-oncogene becomes an oncogene (i.e., a gene able to cause cancer) when a mutation activates it, causing a gain of function. As a result the cell is transformed and proliferative activity enhanced. Of the 100 oncogenes identified so far, few seem to play a key role in human carcinogenesis.

Tumor suppressor genes, on the other hand, normally exert an inhibitory control on the cell cycle. Every mutation leading to loss of function of these genes (for example, a gene deletion) deprives the cell of protection against cancer. Ten tumor suppressor genes have been described to date, and it is speculated that at least six or seven of these genes need to be altered in a single cell before cancer is switched on. This may explain the long latency period between exposure to a certain carcinogen and tumor onset. It also explains higher rates of cancer in older people, as more mutational events occur over time [1, 18].

This review, which focuses on the oncogenes studied in transitional cell carcinoma (TCC), aims to describe their mechanisms of action and to investigate their possible prognostic value.

Oncogene families and their location in the cell

Proto-oncogenes code for proteins located in a defined order throughout the cell. They represent a molecular pathway acting to transduce a proliferative signal from the extracellular space to the nucleus. As shown in Fig. 1, these proteins can be grouped into five families according to their specific localization in the cell.

A normal cell can modulate its proliferative activity via an extracellular signal, a protein usually released by the surrounding cells. This protein, called growth factor, binds to a specific growth factor receptor on the cell membrane. The complex growth factor receptor triggers the activation of a series of membrane-associated proteins. The interaction of the latter group leads to the activation of another series of oncogenic proteins located in the cytosol (cytosolic proteins) which finally carry the message to the nucleus, switching on transcription factors. The proliferative signal transduction from a cell membrane to the nucleus is shown in Fig. 2.

All genes related to these proteins are proto-oncogenes and can undergo oncogenic activation in many human cancers. Figure 1 shows the main proto-oncogenes for each family. Oncogenic activation at any level of the pathway will result in an increased transcription of genes that enhance the cell cycle, resulting in amplified proliferation [35].

Oncogenes coding for growth factors

Growth factors in malignant cells

Malignant cells, unlike normal cells, can grow in culture media deprived of serum. The reason may be that cancer cells provide their own growth factors. This *autocrine* pathway gives proliferative advantage over normal cells in vitro and in vivo.

The most characterized growth factor is epidermal growth factor (EGF). Its main features are listed in Table 1. Transforming growth factor α (TGFα), first isolated from a polypeptide mixture obtained by

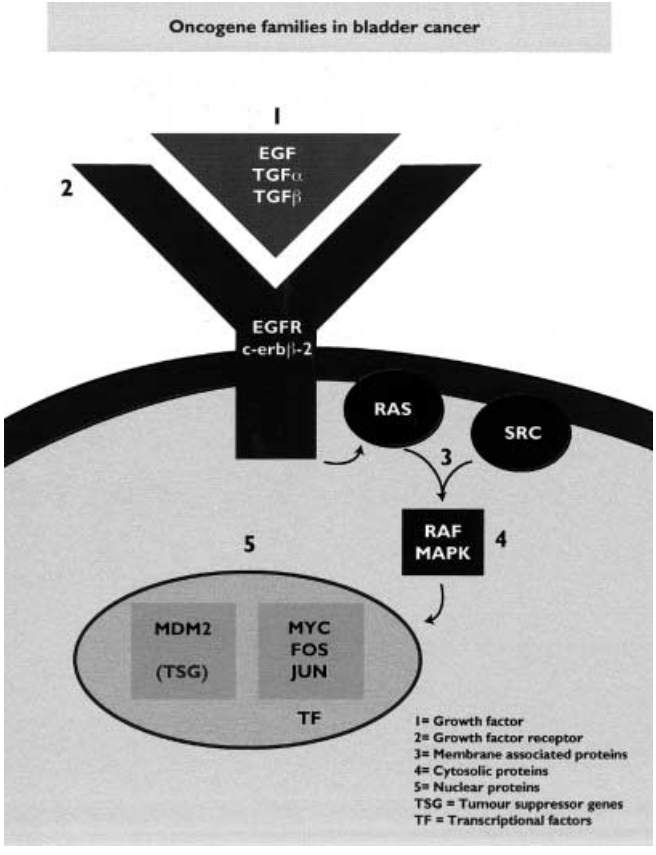


Fig. 1 Extra- and intracytoplasmatic localization of the main oncogenes and tumor suppressor genes involved in bladder carcinogenesis (from Lewin 1996 [35], modified and simplified)

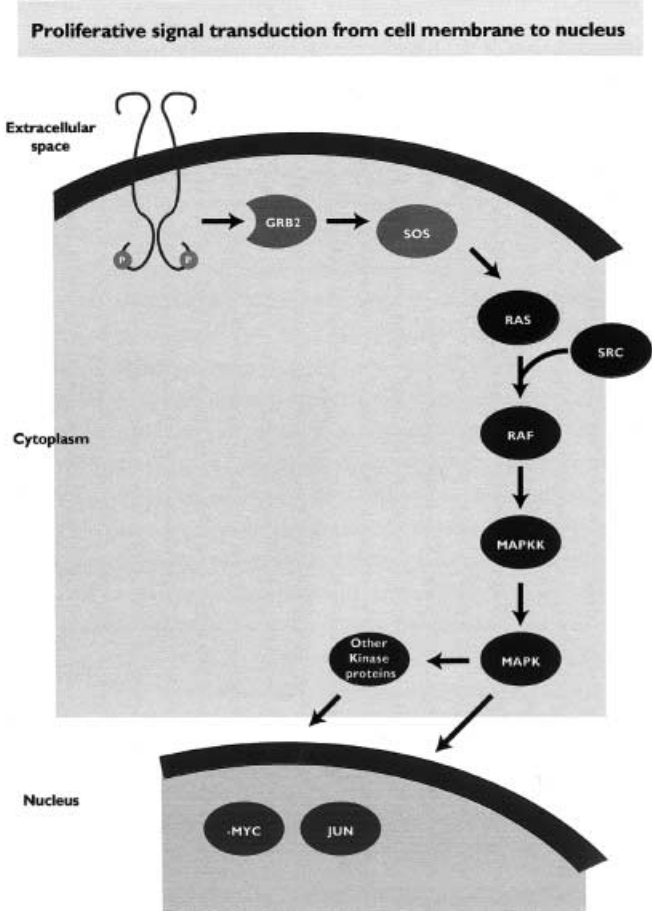


Fig. 2 Pathway of the proliferative signal transduction from the extracellular space to the nucleus (modified from Lewin 1996 [35])

Table 1 Growth factors in transitional cell carcinoma (*TCC*)

Type	Main features	Mechanism of action	Target	Techniques for assessment	Prognostic value
EGF	First in a family of growth factors for EGF	Stimulates proliferation of normal and neoplastic cells [19]	EGFr	IHC	Expressed in nearly 45% of invasive bladder TCCs [73]
	Mitogen activity towards many cell lines				No correlation with grade and stage [30]
	53 amino acid polypeptide				No correlation with survival [73]
	Excreted by several tissues				Strong association with recurrence of superficial TCCs [21]
	High concentrations found in urine of healthy subjects				
TGFα	35% homology with EGF	Stimulates cell growth	EGFr	IHC	Expressed in 60% of invasive bladder TCCs
	Member of the EGF family, binds to the same receptor				Significant correlation with tumoral grade and stage ($P < 0.05$) and with survival ($P = 0.009$)
	Secreted as a precursor polypeptide of 160 amino acids				Independent prognostic value over stage [73]
					Better correlation with EGFr than EGF [59]
					High level of mRNA may predict relapses [46]
					No correlation with survival [22]
TGF β-1, β-2, β-3	Isoforms of the TGFβ family	Mesenchymal cell-growth? stimulator and epithelial cell-growth inhibitor		Northern blotting	TGFβ1-RNA expression decreases with progression in grade [54]
		Promoter of apoptosis		PCR-RNA	TGFβ-1, 2, and 3 expression is reduced in bladder cancer compared with normal urothelium [31]
		Inhibitor of cell proliferation through suppression of c-myc [76]			TGFβ1 levels significantly increases in superficial TCCs compared with invasive ones [31]
		Activator of angiogenesis [33]			Selective loss of expression of β1 isoforms correlates with progression [13]

EGF epidermal growth factor, *EGFr* epidermal growth factor receptor, *IHC* immunohistochemistry, *TGF* transforming growth factor

rodent sarcomatous cells, is known to act as a "foot on the accelerator" towards cell growth. Transforming growth factor β (TGFβ), obtained from the same polypeptide mixture, is an example of a negative growth factor working mainly as a "foot on the brake." [1]. It is possible that the response to TGFβ is lost in advanced cancer, with a positive effect on cell growth [76]. Other possible mechanisms of actions of TGFβ are shown in Table 1 [36].Growing evidence suggests an increased availability of certain growth factors in cancers may reflect the activation of one or more oncogenes [1].

Prognostic value of growth factors in bladder TCCs

Epidermal growth factor

Only limited data on the expression of epidermal growth factor (EGF) in bladder TCCs are available. In 1984 Messing reported a response of cultured bladder cancer cell lines to exposure to EGF from normal rat urine [46]. These results were confirmed using EGF extracted from human urine [32], although EGF concentration in urine from TCC patients had previously been similar to that in a control group [42]. Conversely,

some authors reported lower EGF levels in bladder cancer patients than in healthy people, suggesting a possible consequence of increased binding to an overexpressed epidermal growth factor receptor (EGFr) in cancer cells [21,30]. A strong association between EGF positivity and tumor recurrence has been observed [76], although no correlation between EGF levels and prognosis in bladder cancer was demonstrated in two previous papers [21,59].

Transforming growth factor α

In bladder TCC, EGFr overexpression is often associated with high TGFα levels compared to EGF, leading to the conclusion that TGFα is the main ligand of EGFr [43]. TGFα levels are higher in bladder cancer than normal controls [44]. Immunohistochemical expression of TGFα has proven to correlate well with stage, grade, and survival rates. Simultaneous expression of TGFα and its receptor seems to be a reliable indicator of poor prognosis for bladder cancer [59]. High TGFα mRNA levels have been found to correlate with local relapses in early stage bladder cancer [22].

Transforming growth factor β

TGFβ-RNA is expressed mainly in low-grade and low-stage bladder cancer, while in high grade cancer it is virtually undetectable [31]. RNA concentrations in normal urothelium are even higher [13]. This observation supports the negative control on tumorigenesis of this peptide The inverse correlation with c-myc-RNA (an indicator of oncogene-induced cell growth) suggests inhibition of transcription genes as its possible mechanism of action [33]. Loss of expression of TGFβ receptor has also been observed in bladder cancer, and is associated with increased probability of tumor progression [72].

Oncogenes coding for growth factor receptors

Structure and mechanism of action of a growth factor receptor

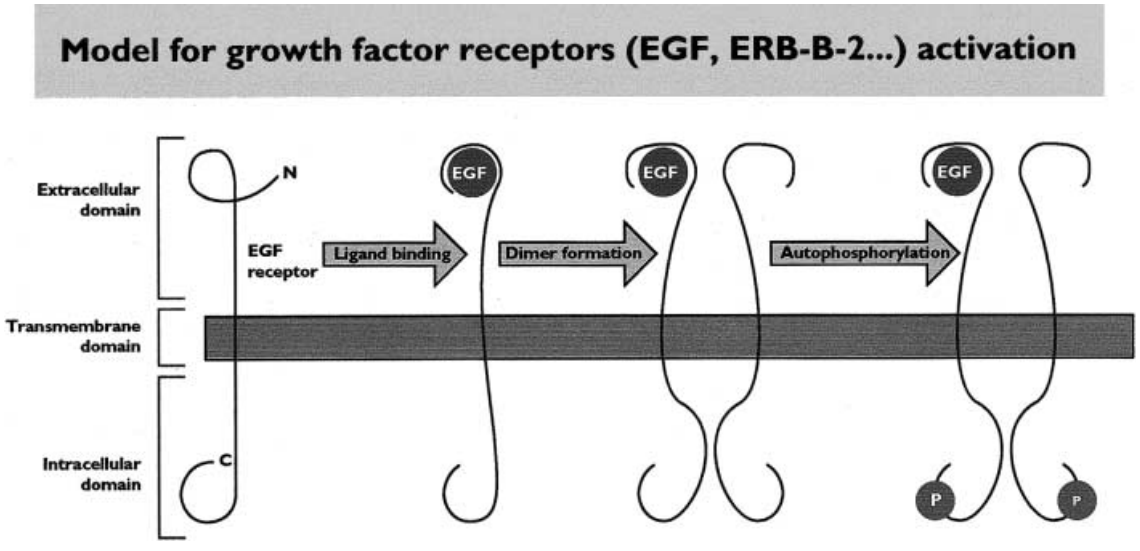
Growth factor receptors are located on the cell membrane and share a common structure consisting of an extracellular domain for the growth factor binding, a short transmembrane domain, and an intracellular portion that initiates the intracellular molecular pathway. The activation of target proteins involves phosphorylation of tyrosine amino acids by a tyrosine kinase enzymatic site located on the intracellular domain of the receptor [35] (Fig. 3). Examples are the EGFr and erbB-2 receptors, expression of which is altered in many human tumors including bladder cancer. EGFr is a 170 kDa glycoprotein coded by the erbB-1 gene [1]. The erbB-2 proto-oncogene is located on chromosome 17q and its protein product has a significant homology with EGFr [80]. TGFα is likely to be the main c-erbB-2 ligand [34].

Figure 2 shows activation of a growth factor receptor. After binding with the ligand the receptor undergoes a dimerization, and the intracellular domains of the two molecules thus formed are put in close relationship. As a result a tyrosine autophosphorylation reaction on the intracellular site occurs. This, in turn, activates the membrane-associated proteins so that signal transduction is guaranteed [35].

Growth factor receptors in cancer

In cancer cells the proliferative signal initiated via the growth factor receptor–growth factor interaction is often amplified. Malignant cells have been shown either

Fig. 3 Phosphorylation of the intracellular domain of the epidermal growth factor receptor (EGFr) following binding to the EGF (from Lewin 1996 [35])



to secrete high levels of growth factors or to increase the number of receptors (receptor up-regulation). EGFr overexpression is a common finding in many human tumors, and amplification or increased transcription of the normal gene, are possible explanations [1]. The erbB-2/neu oncogene, described for the first time in the rat neuroblastoma, is the mutated form of the erbB-2 proto-oncogene (a point mutation results in an increased capacity of the receptor to undergo dimerization). ErbB-2 mutations in human cancers are rare, with gene amplification being the most common finding [11].

Growth factor receptors in TCCs

EGFr in TCCs

Molecular studies. EGFr gene amplification detected by means of Southern blotting (a molecular biology technique that enables isolation of a specific gene from the entire DNA of a cell) is a rare event in bladder TCCs, occurring in one out of 31 cases [3] and one out of 35 patients [23]. Conversely, mRNA overexpression has been found in 36% of cases [78] as a likely consequence of increased gene transcription. Thus, increased numbers of the receptor rather than a mutated receptor appear to be involved.

Immunohistochemical studies

EGFr immunostaining is a common finding in normal urothelium and is normally confined to the basal cell layers [77]. In TCCs, EGFr expression is altered and can be identified throughout the entire cell layers [3,49]. The immunohistochemical expression of EGFr has proven to be a prognostic marker for bladder TCC, as shown in Table 2. A number of studies report a significant correlation between EGFr overexpression and poor prognosis. Exceptions to this are works by Ravery [59] and Sriplakich [68]; both reports, however, only studied invasive bladder tumors.

erbB-2 in TCCs

Molecular studies. Gene amplification, a rare event for EGFr, is a more common finding in c-erbB-2, found in 11% of bladder tumors [23]. In a series of 92 bladder cancers gene amplification was found in 26% of cases and was significantly related to pathological grade and stage [53]. Conversely, Mellon et al. [44] reported an overexpression of the oncogenic protein in 20 out of 95 tumors; however, the presence of multiple copies of the gene was detected in only one case. This observation favors the hypothesis that gene amplification is not the primary mechanism of erbB-2 overexpression.

Table 2 Epidermal growth factor receptor (EGFr) in transitional cell carcinoma (*TCC*): Immunohistochemical studies

Author	Case material	Stage	Correlation with tumor grade and stage	Prognostic value
Neal 1985 [49]	48 bladder TCC	24 superficial	Expression significantly higher in superficial tumors than in invasive ones	
		24 invasive		
Neal 1990 [50]	101 bladder TCC	Mixed superficial and invasive	48% overall expression	Expression level in recurrent forms significantly higher than in nonrecurrent ($P = 0.004$)
			Significant correlation with stage ($P < 0.001$) and with grade ($P < 0.01$)	EGFr is the most powerful predictor of progression (according to Cox)
Wright 1991 [79]	85 bladder TCC	42 superficial	15% with heavy staining, 23% with weak staining	
		33 invasive	Strong correlation with stage and grade when EGFr is simultaneously expressed with p53	
Lipponen 1994 [39]	234 bladder TCC	Mixed superficial and invasive	EGFr immunostaining significantly higher in superficial tumors than in invasive tumors	Prognostic value on progression, not on recurrence
				Prognostic power superior to p53 and erbB-2
Ravery 1997 [59]	41 bladder TCC	Invasive	85% positively stained	No prognostic value on survival
			No correlation with grade and stage	
Sriplakich 1999 [68]	173 ladder TCC	Invasive		No independent predictor of outcome after cystectomy
Liukkonen 1999 [40]	207 bladder TCC	Ta-T1 superficial	–	Significant predictor of progression only with univariate analysis ($P < 0.001$)

Immunohistochemical studies. The role of erbB-2 immunostaining in bladder cancer appears controversial. According to Mellon and coworkers [44], normal urothelium does not stain for the oncogenic protein. On the contrary, Wagner [77], in a report on a large series of premalignant urothelial lesions, found positive erbB-2 expression in normal transitional epithelium restricted to the basal layers; in dysplastic lesions all layers showed positivity. Similar results have come from other units. [37,58].

The studies listed in Table 3 all suggest that erbB-2 overexpression has no prognostic value in TCC. Indeed, according to recent findings [51,59], strong erbB-2 staining in bladder cancers may be related to a good outcome. The authors suggest that loss of expression of erbB-2 oncoprotein may reflect a tumoral switch toward a more aggressive phenotype. Lack of correlation between c-erbB-2 expression and proliferation rate as defined by Ki67 gives support to this hypothesis [48].

ErbB-2 has been investigated in TCCs of the upper urinary tract with disappointing results. Bjerkehagen et al. [4] concluded that immunostaining for this oncoprotein is virtually absent in TCCs of the renal pelvis. Imai [26] suggests a prognostic role, predicting recurrence for a combined erbB-2 and EGFr expression in a series of 30 pelvic TCCs, although no correlation between erbB-2 and pathological grade or stage was seen.

Oncogenes coding for cytoplasmatic proteins

An *ras*-dependent pathway carries proliferative signals from a membrane receptor to the nucleus. The signal

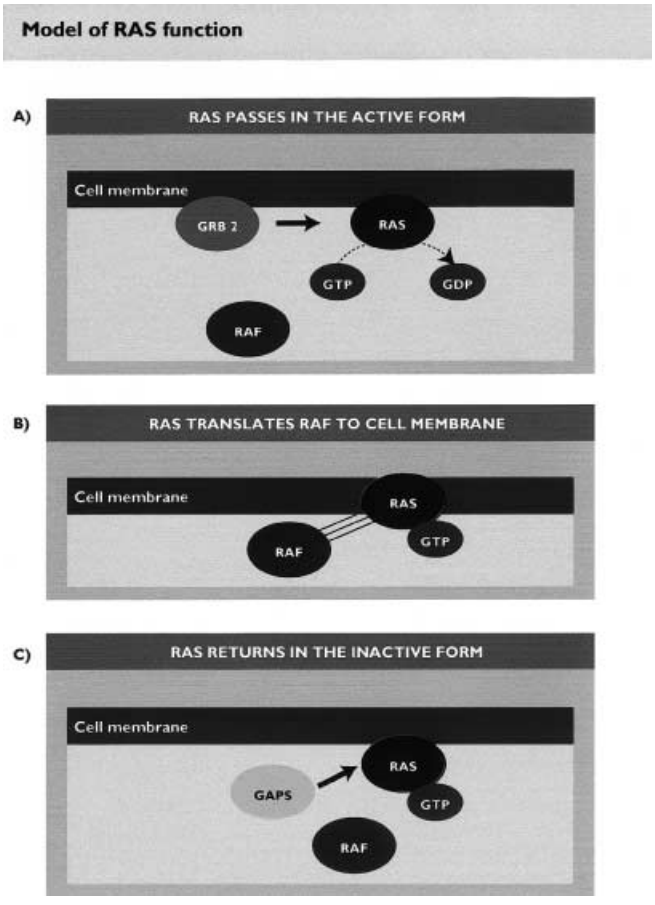


Fig. 4 Model of action of RAS protein:**a** RAS is activated by GTP binding. **b** Activated RAS binds to RAF protein thus allowing its activation. **c** RAS becomes inactivated after GTP is hydrolyzed to GDP by GAPS proteins (from Lewin 1996 [35])

Table 3 ERB-2 in transitional cell carcinoma (*TCC*): Immunohistochemical studies. *EGFr* epidermal growth factor receptor

Author	Case material	Stage/grade	Immunostaining rate and stage/grade correlation	Prognostic value/correlation with other tumor markers
Wright 1991 [79]	82 bladder TCC	Ta–T4	15% overall positivity	No correlation with p53 expression
Swanson 1992 [69]			Weak correlation with stage No correlation with stage	
Lipponen 1993 [37]	249 bladder TCC	Ta–T4	38% overall expression No correlation with stage	No correlation with progression No independent prognostic value on survival No correlation between EGFr and erbB-2 expression
Nguyer 1994 [51]	85 bladder TCC	>T1	29% overall expression	Inverse correlation with survival
Mellon 1996 [44]	95 bladder TCC	Ta–T4	21% with strong staining No correlation with grade and stage	No predictive value on progression and survival
Rajkumar 1996 [58]	65 bladder TCC	G1–G4	8.6% positivity for erbB-2 20% positivity for erbB-3 No correlation with grade for both markers	
Ravery 1997 [59]	42 bladder TCC	>T1	50% positively stained	Inverse correlation with survival No correlation with EGFr

from the receptor is transduced via the proto-oncogenes *ras* and *raf*, which, in turn, activate the proteins involved in the mitogen activated protein kinase (MAP).

Proliferative signal transduction through a cell is carried from an activated growth factor receptor to the nucleus via a series of cytoplasmatic oncogenic proteins. Among these proteins is a family of oncogenes designated *ras*, which have been implicated in a number of models of tumor initiation and promotion. Figure 4 summarizes the main steps of this pathway, defined as *ras*-dependent for the central role played by this oncoprotein in the regulation of the communication between the cell membrane and the nucleus.

A functional model of interaction between these oncogenes suggests that the membrane-associated protein Grb2 binds to an activated growth factor receptor and, at the same time, to the protein SOS, an exchange factor for the guanosine nucleotide. The complex receptor Grb2-SOS is then able to activate RAS. Once RAS becomes activated it interacts with the SRC protein to promote RAF serine/threonine kinase activation. The underlying mechanism of action will be discussed in more detail later. Activated RAF phosphorylates serine and threonine amino acids of MAP kinase, activity of which consists of the phosphorylation in tyrosine and threonine of downstream enzymes – the MAP kinases. These proteins exert a key role in the proliferative signal transduction. MAP kinase, once activated, either interacts with other cytosolic kinase proteins, or translocates inside the nucleus and phosphorylates transcription factors such as the *myc* and *jun* proteins [35].

ras and *ras*-related oncogenes

Physiology

RAS is a family of 21 kDa proteins ($p21^{ras}$) that share the ability to bind the guanosine triphosphate (GTP) and to hydrolyze it to guanosine diphosphate (GDP) in view of an intrinsic GTPase property. The mutated oncogene was isolated for the first time in a retrovirus causing sarcomas in rats (the name derives from rat sarcoma). Three *ras* genes have been defined and are known as c-H-*ras*, c-K-*ras* and c-N-*ras* [1]. When RAS is bound to GDP it is inactive. The Grb2 protein allows substitution of GDP with a GTP molecule, activating the RAS protein. Once activated RAS can exert its function by capturing RAF from the cytoplasm and bringing it close to the cell membrane: this allows RAF activation by the protein $pp60^{c\text{-}src}$. The latter is a membrane-associated oncoprotein coded by the *src* gene, isolated for the first time from the retrovirus causing the Rous SaRComa in chickens.

RAS can be inactivated by intrinsic GTPase activity, as well as by some GTPase activating proteins (GAPs), known for their peculiar feature to accelerate RAS-GTP hydrolysis [1].

RAS activation in cancer

Oncogenic properties of *ras* arise from four hypothetical mechanisms:

1. A point mutation in one of three genes that may reduce intrinsic GTPase activity of $p21^{RAS,}$ thus increasing half-life of the activated protein. Such mutations can be found in nearly 40% of human tumors
2. Inactivation of GAP proteins may prolong RAS activity
3. Hyperstimulation of Grb2 protein by an activated growth factor receptor may stimulate RAS function
4. Overexpression – increased copy numbers of RAS proteins being manufactured by up-regulation of the transcription of a normal *ras* gene

Prognostic value of ras in TCCs

Molecular studies. Mutated *ras* was first isolated from human bladder cancer cell lines employing the transfection technique in mouse fibroblasts [56]. It soon became clear that the oncogene responsible for the transformation of the mouse fibroblasts was almost identical to the c-H-*ras* proto-oncogene found in normal cells [60]. At the same time cell lines containing c-N-*ras* mutations were isolated [5]. Since then numerous molecular studies have been set up to determine frequency and prognostic value of RAS mutations in TCCs. Mutation rate assessed by mean of transfection in the initial studies varied from 7 to 20% [20,57].

Research conducted using the PCR produced disappointing results as regards any prognostic utility of *ras* gene mutations. The main contributions are listed in Table 4. It appears that *ras* mutations involve primarily the H-*ras* gene and are infrequent events in bladder carcinogenesis. Mutation screening in urine sediment has been regarded as a useful tool to increase diagnostic power of urine cytology [16] and is easy to perform [9]; however, the mean incidence of mutations in the few reports available so far is widely variable (from 7 to 76%) (Table 4).

Immunohistochemical studies. An explanation for the immunohistochemical overexpression of p21 protein is still unclear. An increased half-life of the protein coded by a mutated form of *ras* gene, or overexpression of a normal gene product, are possible explanations. In a review of the literature on the prognostic utility of immunohistochemical detection of p21 in human tumors, Gulbis [24] reported that as far as bladder cancer is concerned data are inconclusive. The reason may reside in the contradictory results from early studies where p21 positivity is found in neoplastic as well as normal urothelium [75]. However, later reports identified p21 expression confined to bladder cancer cells [17,71]. Prognostic value of p21 detection in TCCs remains a matter of controversy [12,47,75]. Predictive ability of p21 on tumor recurrence in superficial TCCs

Table 4 Incidence of oncogene RAS mutations in transitional cell carcinoma (*TCC*). *TURBT* transurethral resection of bladder tumor, *PCR* polymerase chain reaction, *RLFP* restricted length fragment polymorphism

Author	Case material	Type of sample	Molecular technique employed	Type of gene	Mutation rate	Site of mutation	Prognostic value
Knowles 1993 [28]	152 bladder TCCs stage Ta–T4	TURBT	RLFP plus sequencing technique	H-RAS	6%	Codons 12, 13, 61	No correlation with grade and stage
Ooi 1994 [52]	62 vescicali stadio Ta-T1	TURBT	Sequencing technique	H-RAS	42%	Codon 12	No prognostic value
Burchill 1994 [6]		TURBT	PCR + sequencing	H-RAS	18%		
Uchida 1995 [74]	8 pelvic TCCs	TURBT	PCR and sequencing	H-RAS	7.7% (3/39)	Codons 12, 13	–
	31 bladder TCCs			K-RAS			
Saito 1997 [62]	50 bladder TCCs	TURBT	PCR + dot blot hybridization	H-RAS	12% (6/50)	Codons 12, 61	
Fitzgerald 1995 [16]	–	Urine sediment			44%		Higher sensitivity compared with urine cytology

remains an isolated finding from recent data produced by Fontana and coworkers [17].

Prognostic value of src in TCCs

C-*src* oncogene has been implicated in the neoplastic development of several human tumors. An enhanced kinase activity of the protein has been linked to de-differentiation of neuroectodermal tumors [61] and to early events of colonic adenocarcinomas [7]. pp60 $^{c\text{-}src}$ expression in TCC was investigated in one study using Southern blotting. Higher levels of the protein were detected in low-grade tumors rather than high-grade tumors [15]. There are no data on the prognostic value of src in bladder cancer.

Oncogenes coding for nuclear proteins (transcription factors)

C-*myc*

Physiology

The name *myc* is derived from its first characterization in the *my*elo*c*ytomatosis retrovirus, known to cause a rare form of myeloid leukemia in chickens. The *myc* gene codes for a 62-kDa protein (p62) [1]. Proliferating cells contain elevated levels of p62. *Myc* protein is known to bind to an 18-kDa protein called MAX, and the resulting DNA-bound complex activates transcription of several transcription factors [2]. Other studies point out the ability of this oncogene to promote not only cell proliferation [14] but also apoptosis [29].

C-myc activation in cancer

Myc becomes carcinogenic when its function is enhanced. In Burkitt's lymphoma this proto-oncogene translocates into the chromosomic region where immunoglobulin (Ig) genes are located, causing a gain in function. Gene amplification seems, however, to be the most common mechanism of *myc* activation in human tumors [35]. Gene modification, as a consequence of altered mechanism of DNA methylation, has been related to tumoral progression. Hypomethylation of *myc* may enhance its transcription activity [55].

C-myc in TCCs

Molecular studies. C-*myc* behavior in bladder TCCs has not been extensively assessed. In solid human tumors c-*myc* function is often enhanced by gene amplification or increased transcription [67]. Schmidt-Drager [66] found a normal number of copies of the gene in bladder cancer culture cells by Southern blotting analysis. However, in a further study from the same group [8], high c-*myc* mRNA levels were related to an increased gene copy number in 15 out of 40 TCCs considered. In a comparative study employing the FISH technique in urothelial tumors, c-*myc* gene amplification was detected in three out of eight cases [65].

Del Senno et al. [76] reported modifications in c-*myc* methylation occurring more often in advanced TCCs. The methylation pattern is well correlated with mRNA overexpression of c-myc [64]. The same author [63] reports a significant correlation between hypomethylation and histological grade.

Immunohistochemical studies. P62 expression in 185 TCCs was 35%, but no correlation was found with pathological stage and prognosis in 175 patients studied by Lipponen [38]. Similar conclusions are drawn in another immunohistochemical study in which p62 protein positivity was 58% [66]. In an earlier report Masters [41] showed that p62 overexpression was associated with tumor recurrence and progression, although protein levels were lower in poor prognosis cases. The method of

Table 5 Oncogenes in clinical practice. *EGF* epidermal growth factor, *TGF* transforming growth factor, *EGFr* epidermal growth factor receptor

Clinical question	Oncogene	Comments
Detection of early disease	Nil at present	We do not know the molecular steps in the initial stages of bladder carcinogenesis
Prediction of recurrence of superficial tumors	EGF TGFα	Superficial tumors expressing these proteins seem to have a high risk of recurrence
Prediction of progression of superficial tumors	TGF-β1	This growth factor seems to protect against progression, possibly indicating stromal inhibition of neoplasia
	EGFr	Seems to be a strong and independent predictor of progression
Prognosis in invasive disease (after cystectomy)	Nil at present	Scattered studies have addressed this question. EGFr has not shown any prognostic utility
Prediction of radiotherapy and/or chemotherapy response	Nil at present	Oncogenes with proven prognostic utility should also be investigated in this respect

investigation used (flow cytometry) makes comparison with recent studies difficult.

C-*jun* and c-*fos*

The c-*jun* and c-*fos* genes function as transcription factors and are characterized by specific DNA binding sites. *Fos* gene product is a 55-kDa phosphoprotein ($p55^{c\text{-}fos}$), expression of which increases only a few minutes after a cell is stimulated. It seems that p55 is able to form a complex with jun protein product to constitute the AP-1 transcription factor [1]. Immunoreactivity for *jun* oncoprotein has been detected in the vast majority of bladder TCCs, but no correlation with traditional prognostic factors has been established [70].

Conclusions

No evidence exists for the routine use of assessment of growth factors or oncogenes in bladder cancer diagnosis or management. Although some may appear promising initially, none have been found superior to conventional grading and staging methods.

In an attempt to enhance the prognostic impact of tumor markers, several oncogenes occupying different key positions in the proliferative cascade have been assessed simultaneously in each tumor sample. Conclusions from various authors are markedly in contrast; however, methodological differences may account for this variability, as is the case in immunohistochemical studies in which various antibodies may differ significantly in their sensitivity as they recognize different epitopes. On the other hand, discrepancies exist among authors on the cut-off values employed to define a positive expression of a certain oncogene.

These drawbacks often make incomparable the results from different studies addressing the same oncogene. It therefore appears advisable that future clinical assessment on a promising oncogenic protein be carried out through multicenter prospective studies conducted after a thorough standardization of methodological variables.

Tumorigenesis is highly dependent upon the loss of function of tumor suppressor genes and not simply the activation of oncogenes. It is possible, by simultaneously testing alterations in both categories of cancer genes, that important clinical information regarding tumor behavior might be obtained. A comprehensive description of tumor suppressor genes was outside the scope of the present study; however, a recent review addressing this topic failed to show a definite prognostic role for any of these genes in bladder cancer [27].

The number of genes found to be closely related to cancer development increases daily. Assessing the pattern of alterations of multiple genes in a single tumor sample using conventional techniques of molecular pathology is slow and time-consuming. Recently a new molecular biology technique, the genes microarray technology, has been developed that detects expression of thousands of genes in the same tumor sample using a single experimental step. In this method DNA probes representing cDNA clones are arrayed onto a glass slide and interrogated with fluorescently labeled cDNA targets in a way that the level of thousands of mRNAs in a tumor sample can be detected [25].

While assessment of growth factors or oncogenes may not yet be a viable method of bladder cancer diagnosis or management, many interesting avenues deserve exploration, and we have listed those we believe to be most promising. Large, long-term studies are needed to evaluate the prognostic value of molecular markers, particularly in cancer subgroups. Possible areas in bladder cancer that could be the subject of further studies are shown in Table 5.

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