Molecular Biology – bladder cancer

Background

Bladder cancer is the most common cancer of the urinary tract and approximately 90% of bladder cancers diagnosed in North America and Europe are transitional cell carcinomas (TCC). For the purposes of diagnosis and treatment, bladder cancer is often classified as either low grade non muscle invasive (LGNMI) or high grade muscle invasive bladder cancer (HGMI), this is also particularly relevant when analysing the molecular pathways involved in the tumourigenesis of these two different phenotypes of bladder cancer. Whilst most muscle invasive bladder cancers are high grade, non-muscle invasive cancer (NMIBC) is far more complex with low, intermediate and high risk NMIBC depending upon grade, size, morphology, multiplicity and presence of CIS. This article describes two distinct pathways but like all tumours, bladder cancer is a heterogenous disease and exists as a spectrum.

Tumourigenesis

Low grade non-muscle invasive cancer

Early tumourigenesis in bladder cancer leads to the development of either a papillary low grade tumour (Ta) that is associated with a high risk of recurrence but a low (5-10%) risk of progression or carcinoma *in situ* (CIS) which has a high risk (approximately 50%) of progressing to muscle invasive bladder cancer if left untreated. The rationale for these two subtypes of bladder cancer remains unclear. Low grade tumours are characterised by increased expression and activating mutations of *FGFR3*, the PI3 kinase pathway and *RAS* [1,2].

FGFR3

FGFR3 is one of the four members of the FGFR family of receptor tyrosine kinases that serve as cell surface receptors for the FGF ligands. Activated *FGFR3* leads to the activation of multiple signalling pathways including the ERK/MAPK cascade and PI3K signalling. Mutations of *FGFR3* have been found in almost 70% of Ta bladder cancers. Up-regulation of *FGFR3* protein expression has been reported in low grade bladder cancer and found to decrease with increasing stage with 80% of pTa tumours, 70% of pT1 tumours and 50% of pT2 tumours exhibiting increased *FGFR3* expression.

PIK3CA

In low grade tumours, the PI3 kinase pathway can be activated by mutations in the p110α catalytic subunit (*PIK3CA*). Mutations in *PIK3CA* have been identified in 15-25% of Ta/T1 tumours [3]. Furthermore, mutations in *PIK3CA* are more common in low grade compared to high grade bladder cancer and these mutations have been shown to occur simultaneously with *FGFR3* mutations [4,5].

RAS

Mutations in the small GTP binding protein RAS have also been reported in around 10% of bladder tumours [6]. Early studies suggested that RAS mutations were more prevalent in low grade Ta/T1 tumours [6], although larger studies are needed to confirm this. Interestingly, mutations in *FGFR3* and *RAS* appear to be mutually exclusive of each other [3,6].

Chromosome 9

In addition, low grade tumours exhibit loss of heterozygosity (LOH) of the long arm of chromosome 9, 9q [7]. A number of tumour suppressor genes are found on chromosome 9q including tuberous sclerosis 1 (*TSC1*), deleted in bladder cancer 1 (*DBC1*) and *PATCHED 1* (*PTCH1*) [8,9]. Further to LOH, mutations in TSC1 have been identified in around 15% of bladder cancer, irrespective of tumour stage or grade [3,5]. These tumours are also associated with a low mitotic or MIK67 antigen ki67 activity.

As mentioned a proportion of these cancers will progress to muscle invasive cancers. Disease progression is associated with abnormalities in chromosomes 8, 11, 13 and 14. The loss of the tumour suppressor genes p53 and pRb are linked to disease progression.

High grade / muscle invasive cancer

In contrast to the mutation spectrum in NMI bladder cancer, MI bladder cancer appear to have frequent mutations in *TP53*, *RB* and *PTEN* genes.

p53

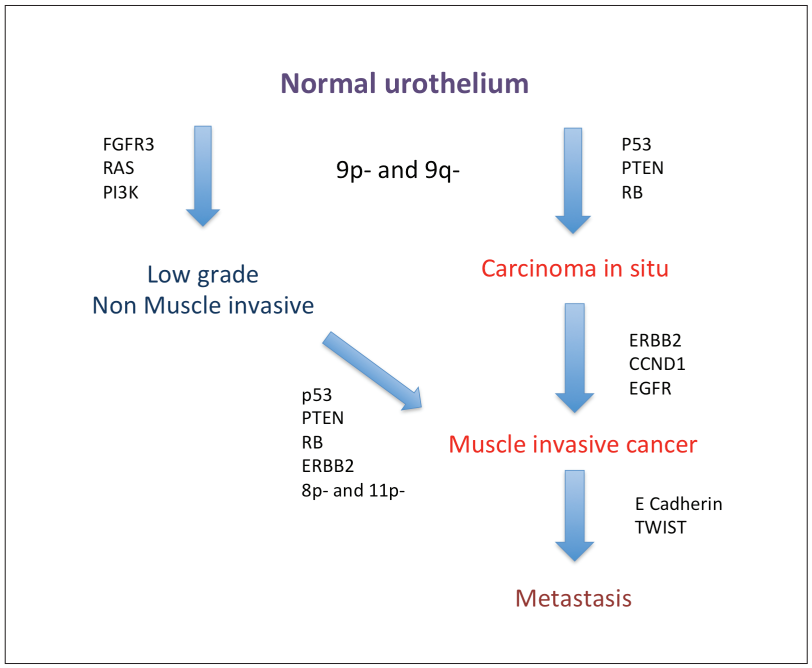
p53 is thought to be integral in the regulation of apoptosis and DNA repair. Deletions or mutations in *TP53* are associated with disease progression and a poor prognosis. Aberrant expression of *p53* has been detected in around 10% of Ta but 60% of T2 bladder cancers suggesting that it is associated with more advanced stage tumours [10,11]. The aberrant expression of other members of the *p53* family has been detected in high grade bladder cancer, reduced *p63* and increased *p73* expression are associated with advanced disease and progression [12].

Retinoblastoma protein (pRB)

pRB is functionally inactivated in a number of solid tumours. *pRB* inhibits cell cycle progression by controlling the exit from the cell cycle into G0/G1. *pRB* is also thought to be involved in DNA replication during S phase and G2/M transition. Deletions of *pRb* have been described in approximately 40% of MI bladder cancers, importantly pRb depletion is associated with deregulation of *E-cadherin* and epithelial to mesenchymal transition.

PTEN

PTEN is known to inhibit the activation of *AKT*, which in turn leads to increased cellular proliferation and inhibition of cell cycle arrest. *PTEN* is linked with an aggressive phenotype and worse prognosis in bladder cancer. The loss of function of these tumour suppressor genes promotes antiapoptotic pathways and activation of a number of known oncogenes. In the majority of cases, the occurrence of *p53* and *FGFR3* mutations are mutually exclusive [10,11]. MI bladder cancers also have a high incidence of loss of heterozygosity of *PTEN* and *Rb*, although the second allele in each case is rarely mutated [13].



ERBB2

Alterations in the transmembrane growth factor receptor *ERBB2*, already acknowledged in poor prognosis breast cancer as a target for therapy, has been shown to be associated with disease progression in bladder cancer through activation of signalling pathways involved in angiogenesis, migration and metastasis.

Metastatic disease

Epithelial-mesenchymal transition (EMT) involves the loss of epithelial cellular characteristics and the development of mesenchymal characteristics such as motility and invasion. Cadherins are cell surface glycoproteins that are involved in cell-cell adhesion. In over 80% of muscle invasive bladder cancers *E-Cadherin* expression is reduced or absent and the majority of invasive tumours show increased expression of *P-Cadherin*, which is associated with absent *E-Cadherin* expression and increasing grade and stage of bladder cancer [14].

TWIST is a transcriptional factor located on chromosome 7p21.2 that inhibits *E-cadherin* and facilitates the shift of cancer cells into the systemic circulation. Aberrant expression of TWIST is associated with inhibition of apoptosis and angiogenesis through increased *VEGF* levels. *TWIST* expression increases with bladder tumour stage and grade suggesting it is involved in bladder cancer progression [15].

Summary

- Loss of heterozygosity of chromosome 9 appears to occur early and be involved in early bladder cancer tumourigenesis.
- There are two distinct pathways in bladder cancer tumourigenesis.
- The low grade papillary phenotype is driven by mutations or altered expression of *FGFR3*, *PI3K* and *RAS*.
- High grade tumours and carcinoma *in situ* are associated with aberrant expression of *p53*, *PTEN* and *Rb*.
- Metastasis is thought to occur through epithelial to mesenchymal transition driven by reduced expression of *E-cadherin* and increased expression of *TWIST*.

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