

Chapter 19

Carcinogenetic Pathway of Superficial Low-Grade Urothelial Carcinoma

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19.1 Introduction

Bladder cancer is a major cause of health expenses. It is estimated to be the seventh most prevalent malignancy worldwide and accounts for approximately 3.2% of the international cancer burden [1]. Bladder cancer is more common in men than in women [2] and more prevalent in industrialized than in developing countries [3].

The bladder is lined by urothelium, so it is no surprise that urothelial carcinoma represents greater than 90% of the tumors within the bladder [4]. Other carcinomas involving the bladder include adenocarcinoma and squamous cell carcinoma. Most cases of urothelial carcinoma are sporadic. Risk factors, such as, cigarette smoking, exposure to arylamines (particularly 2-naphthylamine), *Schistosoma haematobium*

infection, and radiation therapy, have been strongly associated with urothelial carcinoma [5].

Urothelial carcinoma of the bladder is classified into superficial (stage Ta, Tis, and T1) and muscle invasive (T2, T3, T4) tumors. Superficial tumors include non-invasive papillary carcinoma, carcinoma in situ, and tumors that invade the subepithelial connective tissue (lamina propria). They account for 75–85% of urothelial carcinoma of the bladder [6]. More than 70% of patients with superficial tumors will have one or more recurrences after initial treatment [7]. Progression to muscle invasive disease will develop in 10–20% of these patients with superficial tumors [7].

Muscle invasive tumors, which include tumors that invade the muscularis propria, perivesical tissue, and adjacent organs, comprise the remaining 15–25% of urothelial carcinoma of the bladder [6]. Unfortunately, regardless of radical cystectomy and/or systemic therapy, approximately 50% of patients with muscle invasive urothelial carcinoma die from metastases within 2 years of diagnosis [8, 9].

In addition, urothelial carcinoma of the bladder is classified into papillary with low and high grade and non-papillary (flat) tumors. Histologically, urothelial papillary tumors are those that generally consist of fibrovascular cores lined by neoplastic urothelial cells. Low grade papillary carcinoma can have fused papillae lined by predominantly ordered neoplastic urothelial cells that exhibit enlarged nuclei which vary in size and shape. High grade papillary carcinoma demonstrates fused papillae that are branched and lined by neoplastic urothelial cells that show marked variation in size and shape of the nuclei. Non-invasive low grade papillary tumors account for approximately 80% of urothelial carcinoma [10]. These lesions often recur multiple times but are limited in their potential to

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become muscle invasive. The 5 year survival rate is about 90% if these lesions are treated early by surgical resection and intravesical immunotherapy [11].

On the other hand, non-papillary (flat lesions), such as, urothelial carcinoma in situ (CIS), are lesions in which the urothelium contains cells that are cytologically malignant as defined by a neoplastic urothelial cell with a nuclear size of five times or greater than that of a lymphocyte's nucleus. De novo (primary) CIS accounts for about 1–3% of urothelial neoplasms [10] and can present as invasive tumors.

Interestingly, urothelial carcinoma of the bladder is distinct from other epithelial carcinomas in that it is thought to have two divergent pathways of carcinogenesis. Studies have shown that superficial/low grade papillary tumors develop along one molecular pathway while muscle invasive tumors and CIS develop along a different molecular pathway. Deletions of chromosome 9 are more commonly associated with superficial/papillary tumors while loss of heterozygosity on chromosome 17 is more frequently seen in carcinoma in situ and invasive tumors [12–16]. In addition, low grade papillary tumors are shown to have activating mutations involving tyrosine kinase receptors, such as, fibroblast growth factor receptor 3 (FGFR3) [17, 18], and its pathways, such as, Ras (19) and phosphoinositide 3-kinase (PI3K) pathways [19]. In contrast, most CIS and high grade invasive tumors have defects in the p53 and retinoblastoma (RB) protein genes and their pathways [20].

Chromosomal 9 alterations and activating mutations of tyrosine kinase receptors and its pathways involving superficial/papillary tumors will be discussed in this chapter.

19.2 Chromosomal Aberrations

Chromosomal aberrations, which include deletions, amplifications, and aneusomies, are common in urothelial carcinoma and appear to involve almost all the chromosomes [21]. Chromosome 9 monosomy can be seen in non-invasive papillary tumors [22]. However, there are also more localized deletions of various chromosomal regions. Deletions of chromosome 9, although identified in urothelial carcinomas of all grades and stages, is often the only genetic alteration found in low grade tumors [22]. Deletions of both arms of chromosome 9 (9p-/9q-) have been

shown to occur during early urothelial carcinogenesis and are frequently present in superficial low grade papillary tumors [13, 23]. According to Simoneau et al., 48% of superficial tumors had at least one deletion in chromosome 9 [24].

The 9p deletion (9p21) affects the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene. This CDKN2A gene encodes for the tumor suppressor proteins p16 and alternative reading frame (ARF). p16, also known as, inhibitor of cyclin-dependent kinase 4A (INK4A) is a member of the INK4 family. It arrests the G1/S cell cycle transition by preventing the phosphorylation of the retinoblastoma protein (pRB). Loss of p16 expression would, therefore, result in lack of regulatory control of the cell cycle [25].

In addition, loss of heterozygosity of 9q is more common in non-invasive low-grade papillary tumors than in CIS and muscle invasive tumors [13]. Deletions on 9q (9q22.3, 9q31–32, 9q33, and 9q34) are found to be twice as common as deletions on 9p, which so happen to be mostly associated with 9q deletions [26]. This suggests the possibility that gene alterations on 9q may be an early event in superficial papillary tumors [26]. Of interest, even deletions of chromosome 9 are described in normal-appearing urothelium adjacent to areas demonstrating early precursor changes [27]. Furthermore, chromosome 9 deletions are seen in cells taken from voided urine of patients who currently have no detectable tumor and negative urine cytologies [27]. These chromosomal aberrations found in normal-appearing urothelium adjacent to precursor lesions could explain the frequent recurrence of papillary urothelial carcinoma.

However, as previously mentioned, although chromosome 9 deletions may be the only genetic alteration identified in superficial papillary tumors, chromosome 9 deletions have been demonstrated in both urothelial dysplasia and CIS. This would imply that chromosome 9 deletions do not distinguish between the two tumorigenesis pathways [28].

19.3 Activation of Tyrosine Kinase Receptor and Pathway

In addition to chromosomal aberrations, mutations in tyrosine kinase receptors and pathways, such as, FGFR3, PI3KCA, and Ras have been identified in low grade papillary urothelial tumors.

19.3.1 Fibroblastic Growth Factor Receptor 3 (FGFR3)

Typically, when a ligand binds to a cell surface receptor, an extracellular signal is transduced into the cell creating changes in gene expression. Tyrosine kinase is a family of cell surface receptors and consists of an extracellular ligand-binding domain, a transmembrane region, and a cytoplasmic tail that has intrinsic tyrosine kinase activity. Fibroblast growth factor receptor 3 (FGFR3) is a member of the tyrosine kinase family. It is involved in cell growth and differentiation, angiogenesis, and embryogenesis [29]. Specific point mutations in FGFR3 have been associated with human skeletal dysplasias with severe impairment in cranial, digital and skeletal development [30]. Somatic FGFR3 mutations have also been identified in urothelial carcinoma. Seventy percent of low grade papillary non-invasive tumors exhibit FGFR3 mutations [18, 31, 32]. In contrast, only 10–20% of invasive tumors harbor FGFR3 in genes, suggesting that low grade papillary non-invasive tumors have an alternative pathogenesis than invasive tumors [18, 31, 32].

Most of the mutations identified in FGFR3 have been missense mutations that cause amino acid substitutions that involve the extracellular domain, transmembrane region, and cytoplasmic tail [31, 33, 34]. The extracellular ligand-binding domain of FGFR3 consists of three extracellular immunoglobulin-like domains which are connected by loops. The most common mutation results in the conversion of a non-cysteine residue into a cysteine in these loops, with the loop between the extracellular immunoglobulin I and immunoglobulin II being the most common [11]. These mutations can result in autophosphorylation of the intracellular kinase region and decreased translocation to the lysosomal degradative pathway which would could result in increased and prolonged activation of the receptor [35, 36].

19.3.2 Phosphatidylinositol 3 Kinase p110 α (PI3KCA)

Activated FGFR3 can trigger the downstream phosphoinositide 3-kinase (PI3K) pathway. PI3K generates 3'-phosphoinositides which bind to the pleckstrin

homology domain of 3'-phosphoinositides-dependent kinase 1 and Akt with subsequent activation of this pathway [37]. Depending on the substrate specificity, activation mechanisms, and expression patterns, the PI3K family is separated into three classes. Class I is further divided into class 1A subgroup which are coupled to signal transduction by receptor tyrosine kinase upon growth factor binding and class 1B subgroup which signal from G-coupled receptors [38]. Class I PI3K consists of a catalytic (p110) and a regulatory subunit. There are four (α , β , γ , and δ) different catalytic subunits of which the catalytic p110 α subunit is encoded by the PI3KCA locus [38]. Activating somatic mutations in the PI3KCA have been identified in cancers of the breast, colon, ovary, and stomach [39]. Recently, PI3KCA hotspot mutations in codons 542, 545, and 1047, have been found in approximately 20% of superficial bladder tumors in contrast to a very low prevalence in muscle invasive tumors [19]. In addition, a subset of the superficial tumors with PI3KCA has FGFR3 mutations [19]. Therefore, it is quite possible that FGFR3 and PI3KCA may represent a similar pathway of tumor progression. It has been postulated by Lopez-Knowles et al. that activation of PI3K pathway in bladder cancer may enhance malignant behavior in FGFR3-mutant tumors [19].

19.3.3 Ras

In the tyrosine kinase pathway, Ras proteins are also downstream from FGFR3. Ras genes encode membrane-bound guanine nucleotide-binding proteins that are responsible for the transduction of signals that regulate cell growth and differentiation. Ras proteins are activated when bound by GTP and with subsequent hydrolysis of the bound GTP to GDP and phosphate is inactivated. GTP binding can be catalyzed by guanine nucleotide exchange factors. In addition, the rate of conversion from GTP to GDP can be accelerated by guanine nucleotide activating proteins (GAPs). Proto-oncogenes in the Ras family include HRAS, KRAS, RRAS, and NRAS [40].

HRAS was the first human oncogene identified in the bladder cancer cell line T24 [41]. HRAS mutations, which have been found on codons 12, 13 and 61 [42], occur in about 30–40% of low grade non-invasive papillary tumors [43–45]. One specific mutation

frequently found in bladder tumors substitutes the amino acid glycine with amino acid valine at position 12 (G12V) [7]. With this substitution, the HRAS gene is constantly activated which may result in uncontrolled cell division and subsequent tumor formation.

Mutations have not only been found in HRAS but also two other Ras genes, NRAS and KRAS2 [7]. Mutations found in NRAS were G12R, Q61L, and Q61R while mutations found in KRAS2 were G12A and G12V [7]. It is unclear whether both Ras and FGFR3 mutations can co-exist in the same tumor. However, Jebar et al. recently discovered that in no cases were Ras and FGFR3 mutation found together, suggesting mutual exclusion [7].

19.3.4 Other Tyrosine Kinase Receptors

In addition to FGFR3, other tyrosine kinase receptors, such as, the ErbB family can be over-expressed in urothelial carcinoma. The ErbB family includes epidermal growth factor receptor (EGFR or ErbB-1), ERBB2 (HER2/c-neu or c-ErbB-2), ERBB3 (HER3 or c-ErbB-3), and ERBB4 (HER4 or c-ErbB-4). In general, binding of specific ligands leads to dimerization followed by activation of the receptor. Activated receptors are responsible for DNA synthesis and proliferation [46].

Similar to other tyrosine kinase receptors, EGFR is composed of an extracellular ligand-binding domain, a transmembrane region, and an intracellular domain with intrinsic tyrosine kinase activity [47]. Mutations in EGFR may result in persistent activation of the cascades which may lead to uncontrolled cell division [48]. ERBB2 has no external ligand; however, it is believed to be the preferred dimerization partner for other receptors [49]. ERBB3 does not have tyrosine kinase activity, and is therefore, restricted in activation of downstream pathways alone [50]. ERBB4 is more direct in activating the transcription of target genes by moving a portion of its intracellular domain to the nucleus [51].

Interestingly, over-expression of ERBB3 and ERBB4 has been found to be associated with superficial low grade tumors [52]. In contrast, the over-expression of EGFR and ERBB2 are associated with muscle invasive tumors [53–55]. These findings would once again support the two distinct pathways of urothelial carcinoma.

19.4 Conclusion

Even though superficial/low grade papillary tumors are generally are not life-threatening, the disease still places a heavy burden on patients and health-care providers. Following surgical resection of these tumors, patients typically require long-term follow up with invasive procedures. Although their mutual exclusivity is still debatable, tumorigenesis of urothelial carcinoma of the bladder is believed to develop through divergent pathways with division between superficial/low grade papillary tumors and muscle invasive tumors and CIS. With this knowledge, possibly potential markers for non-invasive disease monitoring and for targeted therapy for patients with superficial/low grade papillary tumors may be discovered.

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