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Original article

Mechanistic target of rapamycin (MTOR) protein expression in the tumor and its microenvironment correlates with more aggressive pathology at cystectomy

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Abstract

Background: The mechanistic target of rapamycin (mTOR) has been implicated in driving tumor biology in multiple malignancies, including urothelial carcinoma (UC). We investigate how mTOR and phosphorylated mTOR (pmTOR) protein expression correlate with chemoresponsiveness in the tumor and its microenvironment at final pathologic staging after neoadjuvant chemotherapy (NAC).

Methods: A single-institution retrospective analysis was performed on 62 patients with cT2–4Nany UC undergoing NAC followed by radical cystectomy. Diagnostic (transurethral resection specimens, TURBT) and postchemotherapy radical cystectomy specimens were evaluated for mTOR and pmTOR protein expression using immunohistochemistry of the tumor, peritumoral stroma, and normal surrounding stroma. Protein expression levels were compared between clinical and pathologic stage. Whole transcriptome analysis was performed to evaluate mRNA expression relative to mTOR pathway activation.

Results: Baseline levels of mTOR and pmTOR within TURBT specimens were not associated with clinical stage and response to chemotherapy overall. Nonresponders with advanced pathologic stage at cystectomy (ypT2–4/ypTanyN+) had significantly elevated mTOR tumor staining ($P = 0.006$) and a sustained mTOR and pmTOR staining in the peritumoral and surrounding normal stroma (NS). Several genes relevant to mTOR activity were found to be up-regulated in the tumors of nonresponders. Remarkably, complete responders at cystectomy (ypT0) had significant decreases in both mTOR and pmTOR protein expression in the peritumoral and normal stroma ($P = 0.01$–0.03).

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Conclusions: Our results suggest that mTOR pathway activity is increased in tumor and sustained in its microenvironment in patients with adverse pathologic findings at cystectomy. These findings suggest the relevance of targeting this pathway in bladder cancer.

Keywords: mTOR; Neoadjuvant chemotherapy; Immunohistochemistry; Radical cystectomy; Tumor microenvironment

1. Introduction

The mechanistic target of rapamycin (mTOR) is a major regulator of fundamental biological processes including cell growth and protein synthesis, and is commonly deregulated in human cancers including urothelial carcinoma (UC) [1]. mTOR has been recognized as a cytoplasmic kinase controlling translation, autophagy, and protein degradation [2] but when dysregulated, can be associated with tumorigenesis, disease recurrence, and worsened overall survival [3]. Further, there is evidence that the mTOR pathway can serve as compensatory mechanism for cancers treated with targeted therapies, possessing both cell-autonomous and nonautonomous resistance capabilities [4].

Emerging evidence has demonstrated that the tumor microenvironment is a complex ecosystem of extracellular matrix and stromal growth factors with significant pathway crosstalk and cellular plasticity, each of which may promote tumor progression and treatment resistance [5,6]. One mechanism by which the tumor microenvironment supports tumor growth is the DNA damage response—a complex and coordinated, evolutionary mechanism which allows an organism to repair DNA damage at a constant rate [7]. During UC treatment, however, the genotoxic stress induced by "standard of care," platinum-based chemotherapy impacts both tumor and fibroblasts in the surrounding stroma. This genotoxic stress then liberates the DNA damage secretory program involving cytokines, growth factors, and proteases to augment chemoresistance [7]. Subsequently, malignancies can then utilize alternate signaling pathways, including canonical WNT-signaling [8], as escape mechanisms to abrogate chemotherapeutic response. Interestingly, mTOR inhibition has shown some efficacy in limiting inflammatory cytokines (e.g., IL-6) and NF-kB transcriptional activity, the latter of which is thought to be a main regulator of WNT signaling in response to DNA damage [8,9]. Genomic subtyping of bladder cancer tumors [10,11] has shed insight into treatment sensitivity, yet molecular evidence points toward a "field-change effect," [12] suggesting that targeting the tumor alone may be insufficient and an improved understanding of the tumor microenvironment in UC is required.

In this study, we evaluate mTOR protein expression in both the tumor and its microenvironment in UC, thereby assessing its influence on pathological outcomes and response to neoadjuvant chemotherapy (NAC). We reveal that mTOR protein expression significantly correlates with more aggressive pathology after NAC and is sustained relative to increasing stage strata. In patients who experience a complete response to NAC, we find minimal mTOR protein expression in the tissue stroma. These results support the relevance of mTOR activity in the UC tumor microenvironment.

2. Materials and methods

2.1. Patient population

All patients undergoing radical cystectomy for UC after NAC at a single institution were retrospectively reviewed under an IRB approved study (2003–2011; UW/FHCRC). The analysis was limited to those patients with available pre-NAC, transurethral resection specimens (TURBT) ($N = 62$). We focused on patients with ≥ clinical T2 disease at diagnosis [13]. Pre-NAC samples were acquired from outside and institutional pathology laboratories, followed by matching with final pathologic, radical cystectomy specimens from our institution. Clinical and pathologic variables of the cohort included age, sex, race, histology, chemotherapy regimen, clinical, and pathologic staging. For evaluating the effects of chemotherapy relative to mTOR protein expression, we grouped patients into complete responders (ypT0), partial responders (ypTa/ypTIS/ypT1), and nonresponders (ypT2–4/ypTanyN+) at cystectomy. All staging refers to the AJCC Cancer Staging Handbook, Seventh Edition (2010) [13].

2.2. Tissue microarray construction

Replicate 1-mm cores were taken from tumor, peritumoral, and normal surrounding stromal regions from both TURBT and cystectomy specimens, as defined by a genitourinary pathologist (FVL). For patients experiencing a complete pathologic response (ypT0), fibrosis at the site of the previous diagnostic TURBT was sampled to allow direct comparison of post-NAC tissue. See supplemental methods for full details.

2.3. Immunohistochemical (IHC) analyses

mTOR, pmTOR, and Ki67 expression was graded in a blinded fashion. Signal intensity (0–3: 0 for negative stain, 1 for faint/equivocal stain, 2 for moderate stain, and 3 for intense stain) and percentage of signal coverage (0–100) of each core were scored, and the product of the intensity and