implicated in high grade and advanced stages of bladder cancer but not approved as independent factor for disease progression [Knowles, 2001]. In addition to cytogenetic markers, various genetic markers were also reported to have prognostic value with regard to cancer specific survival such as epidermal growth factor receptor (EGFR) [Nguyen et al., 1994], \( p53 \) [Schmitz-Drager et al., 2000], \( Ki-67 \) [Margulis et al., 2009] and \( HER2/neu \) [Sato et al., 1992]. Furthermore, studies have also shown the association of gene methylation status with the bladder tumor progression rate and increased mortality rate [Catto et al., 2005]. But none of these markers are currently being in clinical use and their validation remains to be confirmed.

Bladder cancer consists of heterogeneous cells which are proposed to be involved in bladder tumorigenesis indicating complex alterations at the molecular level. Detailed molecular insights into bladder cancer biology will allow precise prediction and decision of treatment for improving patient outcome and quality of life. The molecular pathways and the accompanying molecules important in the pathogenesis, recurrence and progression of bladder cancer were investigated for the identification of therapeutic targets. In a recent study, EGFR was proposed to be an attractive target for the treatment of bladder cancer, but a clinical response was observed in only a small proportion of patients. This low response rate of EGFR-targeted therapy did not render this treatment strategy in use [Black et al., 2008]. Another target, fibroblast growth factor receptor (FGFR) 3 was also implicated in bladder cancer after the discovery of frequent activating mutations and showed that it may represent a good therapeutic target [Tomlinson et al., 2009]. These studies also demonstrated that FGFR3 mutants play a role in regulating proliferation, anchorage-independent growth, and clonogenicity but only at low density. The observation of the effects of FGFR inhibition now required to confirm its specificity after which it could be translated into its therapeutic mode [Tomlinson et al., 2009]. Studies have also identified GSK-3\( \beta \) as a positive regulator of proliferation and survival of bladder cancer cells [Naito et al., 2010]. But reports showed detection of weak cytoplasmic staining of GSK-3\( \beta \) in benign bladder tissues also, which limited its applicability as a potential therapeutic target. Exploring mechanistic relationships between the tumor markers and their molecular function will improve our understanding
of bladder cancer-targeted therapy and lead us to additional potential targets to evaluate for use as novel treatment strategies.

For several years, there has been a continuous search for human tumor specific antigens for cancer screening and as potential immunotherapeutic targets. Over the decades, various categories of tumor antigens were found but recently, a new category of tumor antigens has emerged to be a unique class of antigens referred to as cancer-testis (CT) antigens, which could be important antigen targets for cancer immunotherapy. CT antigens were found to be expressed in various human malignancies with restricted expression in normal adult somatic tissues except in the testis [Suri, 2006]. To date, however, several CT antigens have been shown to elicit coordinated humoral and cell mediated responses [Caballero and Chen, 2009]. The generation of immunogenicity in the human host is considered crucial for CT antigens to validate them as potential cancer vaccine targets. Therefore, the highly tissue restricted expression of CT antigens and *in vivo* immunogenicity in cancer patients reflect its potential as promising target molecules for cancer vaccine and serum based biomarkers.

Recent studies have identified and characterized a new member of CT antigen family; sperm associated antigen 9 (SPAG9) and demonstrated its involvement in mitogen-activated protein kinase (MAPK) signaling pathway [Jagadish et al., 2005]. SPAG9 functions as a scaffolding protein involved in c-Jun NH2-terminal kinase (JNK)-signaling module and JNK is known to play important role in cell survival, proliferation and tumorigenesis. *SPAG9* expression was shown to be associated with various malignancies such as epithelial ovarian cancer [Garg et al., 2007], renal cell carcinoma [Garg et al., 2008], breast cancer [Kanojia et al., 2009], cervix cancer [Garg et al., 2009a], thyroid cancer [Garg et al., 2009c] and colorectal carcinoma [Kanojia et al., 2011]. These observations and findings are suggestive of *SPAG9* as potential target for the development of diagnostic and therapeutic interventions.

The management of bladder cancer is a complex and challenging task. Biomarkers for bladder cancer have been intensively screened over the last decade, but despite new findings they are currently not accepted in clinical practice. It has been
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recently proposed that the aberrant expression of CT antigens may be involved in malignant properties such as immortality, migration, invasion and metastasis [Simpson et al., 2005]. In light of these findings, role of SPAG9 expression and its association with various stages and grades of bladder TCC patients and bladder cancer cell lines was investigated. In addition, in vivo immunogenicity studies were also carried out in bladder TCC patients. The present study documented that SPAG9 is expressed in significant proportion of the bladder TCC patients and in bladder cancer cell lines irrespective of clinicopathological characteristics but not expressed in adjacent non cancerous tissue (ANCT) specimens. Humoral response against SPAG9 protein was found in majority of bladder TCC patients. Furthermore, we evaluated the effect of SPAG9 suppression in bladder cancer cells and found that SPAG9 shRNA silencing remarkably inhibited cell proliferation, migration and invasion. Based on these results, SPAG9 may be proposed as a potential molecular target for the development of diagnostic and therapeutic strategies for the bladder cancer management.