ABSTRACT


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Background: Bladder cancer is the 6th most common cancer in the world and the incidence is particularly high in southwestern Taiwan. Although bladder cancer patients have a low mortality rate, long term follow-up with repeated cystoscopy is required due to the high recurrence nature of the tumor. Therefore, a non-invasive detection assay is urgently required for bladder cancer patients. Aberrant promoter hypermethylation which is considered as a hallmark of cancer for over a decade plays an important role in controlling cancer progression. Nevertheless, detection of promoter hypermethylation in bodily fluid has been implicated as a non-invasive and sensitive tool for cancer diagnosis.

Materials and Methods: 100 bladder cancer patients and 9 adjacent normal tissue samples were recruited. For urine voided, 78 samples from bladder cancer patients and 23 from non-cancer individuals were collected. For methylation analysis, 7 bladder cancer patients and 1 primary normal human urothelium were analyzed using illumina human methylation 27K microarray. The role of epigenetic modifications in ZNF671 in bladder cancer cell lines was analyzed by in vitro promoter assay and epigenetic drug treatment. Methylation level of ZNF671 in tissues and urine samples were also analyzed by bisulphite pyrosequencing and quantitative methylation specific PCR (qMSP). Functional analysis of ZNF671 was investigated by soft agar, invasion assay and in vivo tumorigenicity assay.

Results: By using illumina 27K CpG island methylation array, we identified a novel gene, ZNF671 that is hypermethylated in 7 bladder cancer patient samples but not in primary normal human urothelium. In vitro promoter methylation assay and epigenetic treatment confirmed that the expression of ZNF671 is epigenetically controlled in bladder cancer cell lines. Re-expression of ZNF671 in bladder cancer cell lines inhibited cancer growth in soft agar and invasion assay. Re-expression of ZNF671 suppressed tumor growth in nude mice tumorigenicity assay. Interestingly, overexpression of ZNF671 attenuated the expression of stem cell-related genes in bladder cancer. Moreover, bisulphite pyrosequencing demonstrated a significant correlation between higher methylation level of ZNF671 and cancer grade (p < 0.01) and recurrence free survival (p < 0.05)
in bladder cancer patient samples (n = 100). To investigate the diagnostic potential of this novel epigenetic marker in non-invasive cancer detection, quantitative methylation specific PCR (qMSP) was performed in voided urine samples from bladder cancer patients (n = 78) and normal healthy control (n = 23). The sensitivity and specificity of ZNF671 methylation in cancer detection is 39.7% and 95.6% respectively. Combination of ZNF671 and our previously identified epigenetic markers (IRF8, p14 and sFRP1) can further increased the sensitivity to 100% in voided urine samples from bladder cancer patients. **Discussion:** ZNF671 may be a potential tumor suppressor and is epigenetically silenced in bladder cancer. The clinical potential of ZNF671 methylation in non-invasive diagnosis of bladder cancer warrants further investigation.