**SMPD3 methylation and expression as tumor stratification biomarker and clinical outcome prognosticator for clear cell renal cell carcinoma(CCRCC)**

**Method**

DNA methylation profiling data of 418 CCRCC associated samples: 219 CCRCC,199 cancer adjacent were obtained from The Cancer Genome Atlas project (TCGA) with platform of methylation27K[[1](#_ENREF_1)] under which platform 27,578 CpG site in the human genome were covered and fluorescent signals for methylated (Cy5) and unmethylated (Cy3) alleles give methylation level: β= (max(Cy5, 0))/(|Cy3| + |Cy5| + 100) with about 30 replicate bead measurements per locus. All the data were background-corrected and normalised separately with recommended for each platform. Subsequent analyses were conducted in R[[2](#_ENREF_2)]. Differentially hypermethylated CpG sites or genes between groups were identified by fold change(fd>1.25) and t test (P < 0.05). In the first stage, 2856 differential methylated CpG sites were fullfill the criterion of P < 0.05( the pvalue after bonferroni). In the second stage, MBD-Seq data, which have been widely used to discover cancer specific biomarkers[[3](#_ENREF_3), [4](#_ENREF_4)], including 5 CCRCC and 5 cancer adjacents (unpublished) were used to validate the result of differential methylated gene from Methylation27K array. And at last six genes with impoartant biological functions in renal development meanwhile hypermethylation status in CCRCC were selected to be conducted deep validation by pryosequencing in 85 pair tumor and matched controls from Fudan University Shanghai cancer center.

Table 1, Significantly differential methylated genes between ccrcc and adjacent tissues

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| cpgname | genename | pvalue | pvalue.fdr | pvalue.bonferroni | Fold change |
| cg04511534 | GGT6 | 3.46E-110 | 7.21E-107 | 8.65E-106 | 1.891379745 |
| cg22628873 | GGT6 | 1.16E-42 | 4.58E-41 | 2.89E-38 | 1.649480657 |
| cg05127924 | FBXW10 | 2.11E-73 | 5.28E-71 | 5.28E-69 | 1.914547176 |
| cg00891541 | SMPD3 | 5.39E-70 | 1.07E-67 | 1.35E-65 | 1.899386384 |
| cg10556064 | SMPD3 | 2.65E-57 | 2.73E-55 | 6.63E-53 | 1.682782468 |
| cg19297232 | SMPD3 | 5.06E-20 | 4.78E-19 | 1.26E-15 | 1.467510342 |
| cg08519905 | CD9 | 7.57E-63 | 1.06E-60 | 1.89E-58 | 1.906118383 |
| cg07922606 | HIST1H3E | 2.69E-64 | 4.15E-62 | 6.72E-60 | 2.009304856 |
| cg12782180 | LEP | 1.06E-73 | 2.69E-71 | 2.64E-69 | 2.208969818 |
| cg19594666 | LEP | 2.42E-22 | 2.63E-21 | 6.05E-18 | 1.550117434 |

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3. Zhao, Y., et al., *Methylcap-seq reveals novel DNA methylation markers for the diagnosis and recurrence prediction of bladder cancer in a Chinese population.* PLoS One, 2012. **7**(4): p. e35175.

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