**Supplemental Tables**

|  |  |  |  |  |  |  |  |
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| **Supplementary Table S1:** | | | | | |  | |
| **Clinico-pathological parameters in relation to ITIH5 methylation in trainings cohort #1** | | | | | | | |
|
|  | | | ***ITIH5* methylationb** | | | | |
|  | | | ***n****a* | **low** | **high** | **P-value**c | |
|  | | | | | |  | |
| Age at diagnosis | | |  |  |  |  | |
|  | ≤66 years | | 94 | 74 | 20 | 0.369 | |
|  | >66 years | | 89 | 65 | 24 |
| Gender | | | | | |  | |
|  | male | | 150 | 115 | 35 | 0.633 | |
|  | female | | 33 | 24 | 9 |
| Histological tumor graded | | |  |  |  |  | |
|  | low grade | | 23 | 14 | 9 | 0.920 | |
|  | high grade | | 57 | 34 | 23 |
| Tumor staged | | |  |  |  |  | |
|  | pTa | | 63 | 42 | 20 | 0.508 | |
|  | pT1-pT4 | | 36 | 22 | 14 |
| aUrines of cohort #1; bcut-off level PMR=0.35 representig >90% specificity in ROC curve statistic; cFisher’s exact test; dAccording to WHO 2004 classification; Significant P-values are marked in bold face.   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Supplementary Table S2:** | | | | |  | | **Clinico-pathological parameters in relation to ECRG4 methylation in trainings cohort #1** | | | | | | | |  | | ***ECRG4* methylationb** | | | | |  | | ***n****a* | **low** | **high** | **P-value**c | |  | | | | |  | | Age at diagnosis | |  |  |  |  | |  | ≤66 years | 94 | 71 | 23 | 0.969 | |  | >66 years | 89 | 67 | 22 | | Gender | | | | |  | |  | male | 150 | 117 | 33 | 0.084 | |  | female | 33 | 21 | 12 | | Histological tumor graded | |  |  |  |  | |  | low grade | 23 | 15 | 8 | 0.548 | |  | high grade | 57 | 33 | 24 | | Tumor staged | |  |  |  |  | |  | pTa | 62 | 37 | 25 | 0.889 | |  | pT1-pT4 | 36 | 22 | 14 | | aUrines of cohort #1; bcut-off level PMR=0.43 representig >90% specificity in ROC curve statistic; cFisher’s exact test; dAccording to WHO 2004 classification; Significant P-values are marked in bold face. | | | | | | | | | | | | | | | |
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| **Supplementary Table S3:** | | | | | | | | |
| **Primer sequences and PCR conditions for pyrosequencing analyses** | | | | | | | | |
|  | |  | | | | |  | |
| **Primer** | | **Sequence** | | | | | **Anealing temp [°C]** | |
|
| **ITIH5 PCR For** | | 5’-Biotin-TTYGGGAGGTAGTGGGTGTTGGTAAGAA-3’ | | | | | 56 | |
| **ITIH5 PCR Rev** | | 5’-CTCTATTCRAAAAAAAAACATTCCAAACCTAC-3’ | | | | |
| **ITIH5 sequencing** | | 5’-AAAAAAAAACATTCCAAAC-3’ | | | | |  | |
| **ECRG4 PCR For** | | 5’-GGGGAGGGAGAGAGGATTT-3’ | | | | | 56 | |
| **ECRG4 PCR Rev** | | 5’-Biotin-ACCCCATCAAAACCAAAACAACAAAC-3’ | | | | |
| **ECRG4 sequencing** | | 5’-GGGAGGGAGAGAGGATTT-3’ | | | | |  | |
|  | |  | | | | |  | |
| **pre-PCR cycle conditions:** 95°C for 5 min, 40 cycles of 95°C for 20 s, 56°C for 20 s, 72°C for 20 s and a final extension at 72°C for 10 min. | | | | | | | | |
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| **Supplementary Table S4:** | | |
| **Primer sequences and PCR conditions for qMSP analyses** | | |
|  |  |  |
| **Primer** | **Sequence** | **Anealing temp [°C]** |
|
| **qMSP ITIH5 For** | 5’-CAACACAAATAACCCCTACTATACG-3’ | 62 |
| **qMSP ITIH5 Rev** | 5’-TTTTCGGTTTTAGTTTTATTAGAGTCG-3’ |
| **qMSP ITIH5 probe** | 5’-6-Fam-ACGAAACAAACTTAAAATACCTTCTCCCCG-BHQ-1-3’ |
| **qMSP ECRG4 For** | 5’-GAGAGAGGATTTCGGTGGTATTCG-3’ | 66 |
| **qMSP ECRG4 Rev** | 5’-GAATTATCCCTACGTCGCTACCGA-3’ |
| **qMSP ECRG4 probe** | 5’-6-Fam-ACGAAACCCAACGAATAAACGCCGCG-BHQ-1-3’ |
| **qMSP GAPDH For** | 5’-GAGGATATAGTTTGGTTT TG-3’ | 58 |
| **qMSP GAPDH Rev** | 5’-CCTACCTAATAATAATCTTTACTT A-3’ |
| **qMSP GAPDH probe** | 5’-6-Fam-ACTCCAATCCCTAACCCTACCTTT-BHQ-1-3’ |
|  |  |  |
| **qMSP cycle conditions:** | | |
| Amplifications were carried out by using the following profile: 95°C for 10 minutes, 50 cycles at 95°C for 15 seconds, and primer annealing at 58°C to 66°C for 30 seconds. | | |
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| **Supplementary Table S5:** | | |
| **Primer sequences and PCR conditions for MSRE qPCR analyses** | | |
|  |  |  |
| **Primer** | **Sequence** | **Anealing temp [°C]** |
|
| **qPCR ITIH5 For** | 5’-CGCAAAGAAGCATATTGCAC-3’ | 60 |
| **qPCR ITIH5 Rev** | 5’-TAGCTCTGTTCGGGGAGAAG-3’ |
| **qPCR ITIH5 Probe** | 5’-6-Fam-CCCCTCCCACACCCTCCCAT-3’ |
| **qPCR ECRG4 For** | 5’-GAGAGAGGACCTCGGTGGTACT-3’ | 60 |
| **qPCR ECRG4 Rev** | 5’-CGCGGCCGCGGGTTATC-3’ |
| **qPCR ECRG4 Probe** | 5’-6-Fam-CCGCTTGGCCCTCAGCCCTCTGGC-3’ |
| **qPCR NID2 For** | 5’-TCCCCCTCCATGCTCGCTC-3’ | 60 |
| **qPCR NID2 Rev** | 5’-TAGCTTGCTGGGTGGGCCTG-3’ |
| **qPCR NID2 Probe** | 5’-6-Fam-CCTCCAGCCCACTCTCCGCGCCGC-3’ |
|  |  |  |
| qPCR cycle conditions: 94°C for 5 min, 47 cycles of 96°C for 30 s, 60°C for 30 s, 72°C for 60 s. | | |
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