Genome-Wide DNA Methylation Profiles in Renal Tumors of Various Histological Subtypes and Non-Tumorous Renal Tissues

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Key Words

Chromophobe renal cell carcinoma • Clear cell renal cell carcinoma • DNA methylation • Oncocytoma • Papillary renal cell carcinoma • Precancerous condition

Abstract

Objective: The aim of this study is to clarify genome-wide DNA methylation profiles in renal tumors of various histological subtypes. **Methods:** Bacterial artificial chromosome (BAC) array-based methylated CpG island amplification was performed using tissue samples of 17 patients with papillary renal cell carcinomas (RCCs), chromophobe RCCs and oncocytomas, and the results were compared with those from 51 patients with clear cell RCCs. **Results:** Unsupervised hierarchical clustering analysis based on DNA methylation status clustered type 1 and type 2 papillary RCCs into different subclasses. Although chromophobe RCCs and oncocytomas were clustered into the same subclass, the DNA methylation status of 21 BAC clones was able to discriminate chromophobe RCCs from oncocytomas. The number of BAC clones showing DNA methylation alteration in non-tumorous renal tissue from patients with chromophobe RCCs and oncocytomas was smaller than that from patients with clear cell RCCs.

Biphasic accumulation of DNA methylation alterations was observed in non-tumorous renal tissue from all 68 patients, and patients showing such alterations on more BAC clones had a poorer outcome than patients showing them on fewer BAC clones. **Conclusions:** DNA methylation profiles determining the histological subtypes of renal tumors developing in individual patients and/or patient outcome may be already established in non-tumorous renal tissue at the pre-cancerous stage.

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Introduction

Accumulating evidence suggests that not only genetic but also epigenetic alterations play a significant role in human carcinogenesis. DNA methylation alterations are one of the most consistent epigenetic changes occurring during carcinogenesis in various organs: it is known that DNA hypomethylation results in chromosomal instability as a result of changes in chromatin structure, and that DNA hypermethylation of CpG islands silences tumor-related genes in cooperation with histone modification [1–5].

Although the classification of renal tumors is based largely on histology, the World Health Organization classification has introduced genetic alterations as a hallmark corresponding to the histological subtypes of renal tumors, e.g. clear cell renal cell carcinomas (RCCs), the most common histological subtype, are characterized by loss of chromosome 3p and inactivation of the *VHL* gene at 3p25.3 [6]. Moreover, we have reported the genetic clustering of clear cell RCCs based on array-comparative genomic hybridization analysis and the association between genetic clustering on the one hand and clinicopathological tumor aggressiveness or patient outcome on the other [7]. With regard to epigenetic alterations, we have revealed that non-tumorous renal tissue obtained from patients with clear cell RCCs is at the precancerous stage, showing DNA hypo- and hypermethylation in multiple chromosomal regions [8], employing recently developed array-based technology [9], although precancerous conditions in the kidney have been rarely described because non-tumorous renal tissue shows no remarkable histological changes and is unassociated with chronic inflammation and persistent infection with viruses or other pathogenic microorganisms. We have proposed 2 possible scenarios: (a) genome-wide DNA methylation profiles of non-tumorous renal tissue at the precancerous stage are inherited by the corresponding clear cell RCCs developing in individual patients, and (b) DNA methylation alterations at the precancerous stage may be prone to further accumulation of genetic and epigenetic alterations during progression [9–11]. However, to our knowledge, the results of genome-wide DNA methylation analysis have never been reported for histological subtypes of renal tumors other than clear cell RCCs, such as papillary RCCs, chromophobe RCCs and oncocytomas, though the DNA methylation status of several tumor-related genes has been reported separately in such histological subtypes [8, 12, 13].

In the present study, in order to clarify genome-wide DNA methylation profiles during multistage renal tumorigenesis, we performed bacterial artificial chromosome (BAC) array-based methylated CpG island amplification (BAMCA) [14–16] using a microarray of 4,361 BAC clones [17] for papillary RCCs, chromophobe RCCs and oncocytomas, and the corresponding non-tumorous renal tissue. DNA methylation profiles of patients with renal tumors of such histological subtypes were compared with those of patients with clear cell RCCs revealed by the same method.

Materials and Methods

Patients and Tissue Samples

Tumorous tissue and corresponding non-tumorous renal tissue samples were obtained at nephrectomy from 17 patients with primary renal tumors. These patients had not received preoperative treatment and had undergone nephrectomy between 1999 and 2006 at the National Cancer Center Hospital, Tokyo, Japan. The 17 primary renal tumors were histologically subclassified into 4 papillary RCCs including 2 type 1 papillary RCCs (T52 and T53) and 2 type 2 papillary RCCs (T54 and T55), 10 chromophobe RCCs (T56–T65) and 3 oncocytomas (T66–T68) in accordance with the World Health Organization classification (fig. 1a–d) [6]. Tumors in which almost the entire area showed a papillary or tubulopapillary architecture were classified as papillary RCCs, whereas clear cell RCCs in which only a minor component showed a papillary structure were not. The DNA methylation profiles of tumorous tissue and the corresponding non-tumorous renal tissue from these 17 patients were compared with those from 51 patients with clear cell RCCs (T1–T51; fig. 1e) for whom the results obtained by BAMCA had been reported previously [9]. All the patients gave their informed consent prior to inclusion in this study, which was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan.

BAMCA Analysis

High-molecular-weight DNA from fresh frozen tissue samples was extracted using phenol-chloroform, followed by dialysis. Because the DNA methylation status is known to be organ specific [18], the reference DNA for analysis of the developmental stages of renal tumors should be obtained from the kidney, and not from other organs or peripheral blood. Therefore, a mixture of normal renal tissue DNA obtained from 6 male patients and 3 female pa-

Fig. 1. Microscopic views (**a–e, k–p**) and scattergrams of the signal ratios (test signal/reference signal) obtained by BAMCA (**f–j, q–v**) in tumorous tissue (**a–j**) and non-tumorous renal tissue (**k–o, q–u**) from patients with type 1 papillary RCC (**a, f, k, q**), type 2 papillary RCC (**b, g, l, r**), chromophobe RCC (**c, h, m, s**), oncocytoma (**d, i, n, t**) and clear cell RCC (**e, j, o, u**), and normal renal tissue obtained from a patient without any renal tumor (**p, v**). In type 1 (**a**) and type 2 (**b**) papillary RCCs, chromophobe RCCs (**c**), oncocytomas (**d**) and clear cell RCCs (**e**), many BAC clones showed DNA hypo- or hypermethylation (**f–j**). Non-tumorous renal tissue obtained from patients with type 1 (**k**) and type 2 papillary RCCs (**l**), chromophobe RCCs (**m**), oncocytomas (**n**) and clear cell RCCs (**o**) showed no histological changes in comparison with normal renal tissue (**p**) and could not be histologically discriminated from each other. Even in such non-tumorous renal tissue from patients with type 1 (**q**) and type 2 (**r**) papillary RCCs and clear cell RCCs (**u**), distinct DNA hypo- or hypermethylation was already evident when compared with normal renal tissue (**v**). However, the numbers of BAC clones showing DNA hypo- or hypermethylation in non-tumorous renal tissue obtained from patients with chromophobe RCCs (**s**) and oncocytomas (**t**) were significantly smaller than those in non-tumorous renal tissue obtained from patients with clear cell RCCs (**u**) (table 1).

Table 1. The average number of BAC clones showing DNA methylation alterations (DNA hypo- or hypermethylation) in tumorous tissue and non-tumorous renal tissue obtained from patients with renal tumors

	Tumor		Non-tumorous renal tissue	
	average number of BAC clones showing DNA methylation alterations	p ¹	average number of BAC clones showing DNA methylation alterations	p ¹
Papillary RCC (n = 4)	400.5 ± 249.6	0.390	108.0 ± 95.4	0.173
Chromophobe RCC (n = 10)	334.4 ± 139.7	0.167	89.0 ± 48.7	<i>0.041</i>
Oncocytoma (n = 3)	266.7 ± 205.7	0.970	54.0 ± 2.6	<i>0.028</i>
Clear cell RCC (n = 51)	265.3 ± 150.5	–	176.4 ± 138.2	–

¹ Mann-Whitney U test, compared with patients with clear cell RCCs.
p values <0.05, which indicate significant differences, are italicized.

tients without any primary renal tumor was used as a reference for analyses of male and female test DNA samples, respectively.

The genome-wide DNA methylation status was analyzed by BAMCA using a custom-made array (MCG Whole Genome Array-4500) harboring 4,361 BAC clones throughout chromosomes 1–22 and X and Y [17], as described previously [19, 20]. Briefly, 5-μg aliquots of test or reference DNA were first digested with 100 units of the methylation-sensitive restriction enzyme *Sma*I and subsequently with 20 units of the methylation-insensitive *Xma*I. Adapters were ligated to *Xma*I-digested sticky ends, and PCR was performed with an adapter primer set. Test and reference PCR products were labeled by random priming with Cy3- and Cy5-dCTP (GE Healthcare, Little Chalfont, UK), respectively, using a BioPrime Array CGH Genomic Labeling System (Invitrogen, Carlsbad, Calif., USA) and precipitated together with ethanol in the presence of Cot-I DNA. The mixture was applied to array slides and incubated at 43°C for 72 h. The arrays were scanned with a GenePix Personal 4100A (Axon Instruments, Foster City, Calif., USA) and analyzed using GenePix Pro 5.0 imaging software (Axon Instruments) and Acue 2 software (Mitsui Knowledge Industry, Tokyo, Japan). The signal ratios were normalized in each sample to make the mean signal ratios for all BAC clones 1.0. In accordance with previously described criteria [9], in the tumor and the corresponding non-tumorous renal tissue, DNA methylation status corresponding to a signal ratio of <0.67 and >1.5 was defined as DNA hypomethylation and DNA hypermethylation of each BAC clone compared with normal renal tissue, respectively. In our previous study, tumorous tissue and the corresponding non-tumorous renal tissue of 51 patients with clear cell RCCs (T1–T51) were analyzed by the same BAMCA method using the same array and reference DNA (fig. 1j) [9].

Statistics

Two-dimensional unsupervised hierarchical clustering analysis of the renal tumors with various histological subtypes and the BAC clones based on the signal ratios (test signal/reference signal) obtained by BAMCA were performed using the Expressionist software program (Genedata, Basel, Switzerland). Differences in the average number of BAC clones that showed DNA hypo- or hypermethylation between the histological subtypes of tumors were analyzed using the Mann-Whitney U test. BAC clones whose

signal ratios differed significantly between chromophobe RCCs and oncocytomas were identified by the Wilcoxon test ($p < 0.01$). Survival curves of patient groups were calculated by the Kaplan-Meier method, and the differences were compared by the log-rank test. Differences at $p < 0.05$ were considered significant.

Results

Genome-Wide DNA Methylation Profiles of Renal Tumors

Example scattergrams of the signal ratios (test signal/reference signal) for tumorous tissue from each patient with type 1 papillary RCC, type 2 papillary RCC, chromophobe RCC and oncocytoma, respectively, are shown in figure 1f–i. The average numbers of BAC clones showing DNA hypo- or hypermethylation in papillary RCCs, chromophobe RCCs and oncocytomas were not significantly different from those in clear cell RCCs (table 1).

Figure 2 shows the results of 2-dimensional unsupervised hierarchical clustering based on the signal ratios obtained by BAMCA for 4 papillary RCCs (T52 and T55), 10 chromophobe RCCs (T56–T65), 3 oncocytomas (T66–T68) and the previously examined 51 clear cell RCCs (T1–T51). Two type 1 papillary RCCs (T52 and T53) and 2 type 2 papillary RCCs (T54 and T55) were clustered into the same subclasses and the 2 types of papillary RCCs were clustered into subclasses different from each other, and each accompanied clear cell RCCs.

All 10 chromophobe RCCs (T56–T65) and 3 oncocytomas (T66–T68) were clustered into the same subclass and excluded any tumor of other histological subtypes. On the other hand, the Wilcoxon test ($p < 0.01$) revealed that the signal ratios of 21 BAC clones differed significantly between chromophobe RCCs ($n = 10$) and oncocy-

Fig. 2. Two-dimensional unsupervised hierarchical clustering analysis based on BAMCA data (signal ratios) for tumorous tissue obtained from patients with type 1 (T52 and T53) and type 2 (T54 and T55) papillary RCCs, chromophobe RCCs (T56–T65), oncocytomas (T66–T68) and clear cell RCCs (T1–T51). The signal ratio is shown as color range maps. The cluster trees for patients and BAC clones are shown at the top and left of the panel, respectively.

tomas ($n = 3$). Figure 3 shows scattergrams of the signal ratios in chromophobe RCCs and oncocytomas for representative examples of the 21 BAC clones. In all 21 BAC clones, using the cutoff values of the signal ratios described in figure 3 and table 2, chromophobe RCCs in this cohort were discriminated from oncocytomas with a sensitivity and specificity of 100%.

Genome-Wide DNA Methylation Profiles of Non-Tumorous Renal Tissue Obtained from Patients with Renal Tumors

In our previous study, many BAC clones showed DNA hypo- or hypermethylation even in non-tumorous renal tissue obtained from patients with clear cell RCCs (fig. 1u) when compared with normal renal tissue obtained from patients without any renal tumor (fig. 1v) [9], although non-tumorous renal tissue obtained from patients with clear cell RCCs (fig. 1o) showed no histological changes in comparison with normal renal tissue (fig. 1p). Non-tumorous renal tissue obtained from patients with papillary RCCs (fig. 1k, l), chromophobe RCCs (fig. 1m) and oncocytomas (fig. 1n) did not show any histological changes when compared with both non-tumorous renal tissue obtained from patients with clear cell RCCs (fig. 1o) and normal renal tissue (fig. 1p). Furthermore, there were no histological differences among non-tumorous renal tissue obtained from patients with papillary RCCs (fig. 1k, l), chromophobe RCCs (fig. 1m) and oncocytomas (fig. 1n). However, the average numbers of BAC clones showing DNA hypo- or hypermethylation in non-tumorous renal tissue obtained from patients with chromophobe RCCs and oncocytomas were significantly smaller than the average number in non-tumorous renal tissue obtained from patients with clear cell RCCs (table 1).

A histogram showing the numbers of BAC clones with DNA hypo- and hypermethylation in non-tumorous renal tissue from all 68 patients with renal tumors is shown in figure 4a. Biphasic accumulation of DNA methylation alterations was evident, with a trough of 250 BAC clones in non-tumorous renal tissue. Thus, the 68 patients were divided into 2 groups according to the number of BAC

ly. Two type 1 papillary RCCs (T52 and T53) and 2 type 2 papillary RCCs (T54 and T55) were each clustered into the same subclasses. Type 1 and type 2 papillary RCCs were clustered into subclasses different from each other, and each was accompanied by clear cell RCCs. All 10 chromophobe RCCs and 3 oncocytomas were clustered into the same subclass, which did not include any tumor of other histological subtypes.

Fig. 3. Scattergrams of the signal ratios in chromophobe RCCs (Chr, T56–T65) and 3 oncocytoas (Onc, T66–T68) for representative BAC clones, RP11-140B17, RP11-678G14 and RP11-404P16. Using the cut-off values (CV) described in each panel, chromophobe RCCs were discriminated from oncocytoas with a sensitivity and specificity of 100%.

Fig. 4. a Histogram showing the number of BAC clones with DNA hypo- or hypermethylation in non-tumorous renal tissue obtained from patients with papillary RCCs, chromophobe RCCs, oncocytoas and clear cell RCCs. Biphasic accumulation of DNA methylation alterations with a trough of 250 BAC clones (arrow) was observed in non-tumorous renal tissue. **b** Kaplan-Meier survival curves of patients with papillary RCCs, chromophobe RCCs, oncocytoas and clear cell RCCs. The period covered ranged from 63 to 2,801 days (mean 1,612). The recurrence-free survival rate of patients showing DNA hypo- or hypermethylation on ≥ 250 BAC clones in their non-tumorous renal tissue ($n = 16$, black line) was significantly lower than that of patients showing DNA hypo- or hypermethylation on < 250 BAC clones in their non-tumorous renal tissue ($n = 50$, gray line; $p = 0.0204$).

clones showing DNA hypo- or hypermethylation in their non-tumorous renal tissue (≥ 250 BAC clones vs. < 250 BAC clones). Figure 4b shows the Kaplan-Meier survival curves of 66 patients who underwent curative resection of their renal tumors. The period covered ranged from 63 to 2,801 days (mean 1,612). The recurrence-free survival rate of patients showing DNA hypo- or hypermethylation on ≥ 250 BAC clones in their non-tumorous renal tissue was significantly lower than that of patients showing DNA hypo- or hypermethylation on < 250 BAC clones ($p = 0.0204$; fig. 4b).

Discussion

In tumors of many organs, an association between specific DNA methylation profiles and various histological subtypes has been reported [21, 22]. Such an association may reflect an epigenetic pathway of tumorigenesis, which is specific to each histological subtype. Although

various histological subtypes of tumors occur in the kidney, to our knowledge, there has been no reported genome-wide DNA methylation analysis of such histological subtypes other than clear cell RCC.

Many researchers in the field of cancer epigenetics have used promoter arrays to identify the genes that are methylated in cancer cells [23–25]. However, the promoter regions of specific genes are not the only target of DNA methylation alterations in human cancers. DNA methylation status in genomic regions that do not directly participate in gene silencing, such as the edges of CpG islands, may be altered at the precancerous stage before the alterations of the promoter regions themselves occur [26]. Genomic regions in which DNA hypomethylation affects chromosomal instability may not be contained in promoter arrays. Moreover, aberrant DNA methylation of large regions of chromosomes, which are regulated in a coordinated manner in human cancers due to a process of long-range epigenetic silencing, has recently attracted attention [27]. Therefore, we used a custom-made BAC

Table 2. Twenty-one BAC clones which were able to discriminate chromophobe RCCs (Chr) from oncocytomas (Onc)

BAC clone ID	Location	Cutoff value	DNA methylation status ¹
RP11-201O14	1p34.3–1p36.13	0.950	Chr<Onc
RP11-89O18	1p33–1p34.2	1.070	Chr<Onc
RP11-542D13	2q11.1–2q11.2	0.850	Chr<Onc
RP11-124O2	3p21.2	0.610	Chr>Onc
RP11-745L2	3q13.13d	1.135	Chr<Onc
RP11-89F1	5q32	0.950	Chr<Onc
RP11-79J23	6p21.2–6p21.3	1.070	Chr<Onc
RP11-75C8	6q21–6q22.1	0.920	Chr<Onc
RP11-10D8	7q22.1	0.780	Chr>Onc
RP11-140B17	10q25.3–10q26.13	1.030	Chr<Onc
RP11-196E1	11q23	0.910	Chr>Onc
RP11-170D9	14q11.2–14q12	0.920	Chr>Onc
RP11-91J13	14q23	1.000	Chr>Onc
RP11-397B22	16p13.3a	0.960	Chr>Onc
RP11-122P17	16q24	0.850	Chr>Onc
RP11-798B19	19p	1.050	Chr>Onc
RP11-678G14	19p12b–19p12c	0.650	Chr>Onc
RP11-46I12	19q12–19q13.1	0.650	Chr>Onc
RP11-446K10	19q13.1–19q13	0.950	Chr>Onc
RP11-10D18	20q13.1–20q13.2	0.720	Chr>Onc
RP11-404P16	Xp11.2–Xp11.3	0.900	Chr>Onc

¹ Chr<Onc = when the signal ratio was lower than the cutoff value, the tissue sample was considered to have originated from chromophobe RCC; Chr>Onc = when the signal ratio was higher than the cutoff value, the tissue sample was considered to have originated from chromophobe RCC.

array [17] that may be suitable not for focusing on specific promoter regions, but for overviewing the DNA methylation status of individual large regions among all chromosomes.

Since microscopic observation frequently revealed a papillary RCC component associated with the concomitant clear cell RCC component in a single renal tumor, papillary RCCs and subclasses of clear cell RCCs may have been grouped into the same subclass in the present unsupervised hierarchical clustering (fig. 2). First, based simply on cytologic and histologic criteria, papillary RCCs were divided into 2 morphologic groups, type 1 and type 2. Type 1 papillary RCCs consist of papillae covered with a single or double layer of small cuboid cells with scanty cytoplasm, and type 2 papillary RCCs consist of papillae covered by large eosinophilic cells arranged in an irregular or pseudo-stratified manner [6]. Although type 2 papillary RCC is frequently at an advanced stage

at initial diagnosis, thus resulting in poor patient survival [28], only a small number of molecular differences between type 1 and type 2 papillary RCCs, such as the level of expression of vascular endothelial growth factor receptor and copy number alterations on chromosomes 1p, 3p, 9p and 17, have been reported to date [29]. The present results (fig. 2) indicate that genome-wide DNA methylation profiles may explain the differences in background characteristics between type 1 and type 2 papillary RCCs, although further confirmation in a larger cohort will be needed.

In the present unsupervised hierarchical clustering based on BAMCA data, chromophobe RCCs and oncocytomas formed a subclass by themselves (fig. 2). Histopathological observations have underlined the similarities between chromophobe RCCs and oncocytomas. Since both of these neoplasms consist of tumor cells with abundant eosinophilic cytoplasm and mainly show a solid structure [6], differential diagnosis between them frequently becomes difficult even for experienced pathologists. Both chromophobe RCCs and oncocytomas have been described in patients with Birt-Hogg-Dubé syndrome, which is characterized by cutaneous fibrofolliculomas, renal tumors, pulmonary cysts and spontaneous pneumothorax. Moreover, such patients sometimes develop so-called hybrid oncocytic tumors with histological features similar to both chromophobe RCCs and oncocytomas [30]. On the other hand, the genetic status of chromophobe RCCs and oncocytomas differs markedly: copy number alterations on various chromosomes are frequent in chromophobe RCCs, but are rare in oncocytomas [31]. The present results indicate that similarities of genome-wide DNA methylation profiles may epigenetically cover the genetic differences between chromophobe RCCs and oncocytomas and may be able to explain the phenotypic similarities of these tumors. On the other hand, regional DNA methylation alterations on the 21 BAC clones were able to discriminate chromophobe RCCs and oncocytomas (fig. 3; table 2). Even though the overall epigenetic pathway of tumorigenesis may be shared by chromophobe RCCs and oncocytomas, there may be target chromosomal regions of DNA methylation alterations that are specific to each neoplasm. In addition to copy number status, the DNA methylation status in such chromosomal regions may become a hallmark for differential diagnosis of these morphologically similar tumors.

Surprisingly, the DNA methylation status (the number of BAC clones showing DNA hypo- or hypermethylation) of non-tumorous renal tissue obtained from chro-

chromophobe RCCs and oncocytomas was significantly different from that of clear cell RCCs, suggesting that histological subtype-specific DNA methylation alterations have already occurred, even in apparently normal renal tissue (table 1). Although we analyzed samples of non-tumorous renal cortex tissue as well as tumorous tissue using normal renal cortex tissue as a reference for the comparison of all histological subtypes (table 1), chromophobe RCCs and oncocytomas are considered to be derived from the intercalated cells of the collecting duct. We also examined DNA methylation status in non-tumorous renal medulla tissue obtained from patients with chromophobe RCCs using a mixture of normal renal medulla DNA as a reference. The numbers of BAC clones showing DNA methylation alterations in renal medulla tissue did not differ significantly from those in renal cortex tissue obtained from individual patients with chromophobe RCCs (online supplementary table 1, www.karger.com/doi/10.1159/000322072). Therefore, we were able to observe differences of DNA methylation status between non-tumorous renal tissue from patients with chromophobe RCCs and that from patients with clear cell RCCs even when we used tissue samples of the renal medulla and cortex, which are the tissues of origin of chromophobe RCCs and clear cell RCCs, respectively. It is possible that the DNA methylation status of non-tumorous renal tissue obtained from patients with papillary RCCs was not different from that of clear cell RCCs (table 1), because papillary RCCs themselves showed DNA methylation profiles similar to those of clear cell RCCs (fig. 2).

It is known that patients with chromophobe RCCs and oncocytomas generally show a more favorable outcome than patients with clear cell RCCs [32]. Since patients

with chromophobe RCCs and oncocytomas showed DNA methylation alterations in non-tumorous renal tissue that differed from those of patients with more aggressive clear cell RCCs (table 1), we evaluated the correlation between the DNA methylation status of non-tumorous renal tissue and patient outcome. Surprisingly, patients with accumulation of DNA methylation (DNA hypo- or hypermethylation on ≥ 250 BAC clones) in their non-tumorous renal tissue showed a poorer outcome than patients without such accumulation (DNA hypo- or hypermethylation on < 250 BAC clones; fig. 4b). Although one cannot easily conclude that DNA methylation alterations in non-tumorous renal tissue are correlated with histological subtype (chromophobe RCCs and oncocytomas vs. clear cell RCCs) or patient outcome (favorable outcome vs. poorer outcome), or both, the present study including various histological subtypes indicated that DNA methylation status was not simply altered in precancerous conditions, but that significant DNA methylation profiles determining the histological subtypes of future developing renal tumors and/or patient outcome are already established at the precancerous stage.

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