Chromosome 7 aneuploidy in clear cell and papillary renal cell carcinoma: Detection using silver in situ hybridization technique

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Abstract

Background: Chromosome 7 aberrations in renal cell carcinoma (RCC) have been reported in papillary renal cell carcinoma (pRCC) and clear cell renal cell carcinoma (ccRCC). However, the implication of these anomalies on prognosis and survival is still unclear. RCC Chromosome 7 aberrations have commonly been detected by fluorescent in situ hybridization and chromogenic in situ hybridization but not silver in situ hybridization (SISH). Aim: The purpose was to report chromosome 7 aberrations in ccRCC and pRCC using SISH in paraffin-embedded tissues and determine the association between the anomalies with clinical and pathological features. Materials and Methods: Cases of ccRCC and pRCC from University Malaya Medical Centre (2001-2009) were analyzed. Chromosome 7 staining was performed using an automated SISH method and association tests between chromosomal anomalies, clinical features and survival were performed. Results: SISH is a feasible technique to detect chromosome 7 aberration in RCC. Chromosome 7 aberrations with nuclear grading, staging and survival yielded no significant correlation. Surprisingly, there was a significant association between gender and chromosome 7 expressions. Though grade did not reach statistical significance for survival in our RCC cases, there...
was a significant correlation between overall survival with race and stage. **Conclusion:** Chromosome 7 aberrations in ccRCC showed no prognostic significance. Nevertheless, staging and grading systems that include prognostic variables could hold better promise.

**Keywords:** Chromosome 7, monosomy, polysomy, renal cell carcinoma, silver in situ hybridization

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**Introduction**

Renal cancer accounts for 2% of all human cancers and renal cell carcinoma (RCC) is the most common form of renal malignancy. RCC consists of several subtypes, each with its own histopathological and genetic characteristics.

About 75% of renal carcinoma is of clear cell renal cell carcinoma (ccRCC) subtype. [1] papillary renal cell carcinoma (pRCC) represents the largest subgroup among the non ccRCC classifications. [2] It is well-known that specific chromosome abnormalities occur in subtypes of RCC such as deletion of 3p in ccRCC and trisomies of chromosome 7 and 17 in pRCC. [3][4][5] Duplication of chromosome 7 is found in 75% of papillary cases. [6] Chromosome 7 gain is more commonly reported in type 1 pRCC, which is mainly hereditary compared with the sporadic variant usually found in type 2 pRCC. As type 1 pRCC is usually of lower histological grade than type 2, trisomy 7 is present more often in low-grade compared with high-grade pRCC. [7][8] Chromosome 7 abnormality is not exclusive to the papillary subtype as it has also been reported in ccRCC. [9] However, the significance of this aberration as a prognostic factor in ccRCC is unclear.

Chromosome 7 aberration in RCC has been detected using techniques such as fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH) and G-band karyotyping, but not silver in situ hybridization (SISH). [10][11][12] The aims of the present study were to report chromosome 7 aneuploidy in ccRCC using SISH technique in paraffin embedded tissue and to determine the relationship between the anomalies and clinical prognosis and pathological features.

**Materials and Methods**

**Patient tissue samples**

Paraffin embedded sections of histologically diagnosed cases of ccRCC and pRCC [Figure 1] between 2001 and
2009 from the Department of Pathology, University of Malaya were analyzed. Representative paraffin embedded blocks of the tumor was selected. The pathological features recorded included Fuhrman nuclear grading (4 tiered Fuhrman nuclear grading) and tumor node metastasis (TNM) staging based on the tumor size, node involvement and metastases. Clinical features and survival data were obtained from patients' records. Ethical approval was attained from the institutional review board.

Figure 1: Silver staining of chromosome 7 in clear cell renal cell carcinoma nuclei. The clear arrows show normal chromosome 7 copy numbers (diploid). Black arrows indicate nuclei with (a) trisomy 7 and (b) monosomy 7: (Silver in situ hybridization, ×40)

Chromosome 7 SISH

SISH was performed on 5 μm thick paraffin sections of the tumors using an automated Ventana Benchmark instrument (Ventana Medical Systems, Tucson, AZ). Slides were stained according to the manufacturer's protocols for the INFORM Chromosome 7 Probe (Ventana) and ultraView™ SISH Detection Kit (Ventana). The silver deposition of chromosome 7 was visualized as a black dot [Figure 1]. Photographs of 20 fields under the high power (40 magnifications) were taken for each patient slide using an Olympus microscope camera.

Signals in the nuclei were counted and overlapping nuclei were not taken into account. The hybridization data was analyzed based on chromosome index (CI) and signal distribution as described by An et al. CI was calculated by dividing the total number of hybridization spots by the total number of nuclei. The signal distribution represented the percentages of the nuclei with 1, 2, 3 or >3 hybridization spots. Two copies per nucleus demonstrate normal chromosomal (diploid) pattern, one copy per nucleus is monosomy while polysomy is characterized by the detection of three or more signals per nucleus. A tumor was regarded as monosomy for chromosome 7 if the CI was less than the mean -3 standard deviation (SD) of control or if the percentage of nuclei with one hybridization spot was more than the mean +3 SD of the control. A tumor was considered as polysomy if the CI was more than the mean +3 SD of the control.

Statistical analysis

Statistical analysis was carried out using the SPSS version 16.0 (SPSS, USA). Association tests between chromosomal ploidy and gender, age, race, RCC type, grade or stage were performed using the Chi-square or analysis of variance test and survival analysis was carried out using the Kaplan-Meier analysis. A \( P < 0.05 \) was considered as statistically significant.

Results

Demographics and pathological features

A total of 40 cRCC and 3 pRCC tumor slides were analyzed [Table 1]. There was a 2.1:1 male to female ratio for the RCC cases. The three main ethnic groups of Malaysian population are Malays, Chinese and Indians. The majority of
the analyzed RCC tumors here were Chinese at 48.8% while Malays were 27.9% and Indians, 23.3%. Patients were most frequently diagnosed at Stage I with 65.1%, followed by 20.9% for Stage II, Stage IV 11.6% and Stage III at 2.3%. The tumors were mostly graded as Fuhrman nuclear Grades II (44.2%) and III (41.9%).

Table 1: Patient demographics, clinicopathological data and chromosome 7 copy number

Chromosome 7 monosomy and polysomy

The mean CI of the control kidneys was 1.66 ± 0.08 and the percentage of nuclei with one signal was 34.8 ± 7.8%. Therefore, tumors with the CI of more than 1.87 were considered polysomy 7 while tumors with the CI of less than 1.44 or more than 57.5% of nuclei containing only one signal were regarded as monosomy 7. Among the ccRCC tumors, 22.5% was monosomy and 12.5% was polysomy. None of the pRCC was monosomy, but 33.3% was polysomy [Table 1]. All 6 cases of polysomy tumors were males. There were 3/29 (10.3%) monosomy tumors in males while in females, 6/14 (42.9%) of the tumors were monosomy. There was a significant difference in the distribution of monosomy and polysomy cases in relation to gender. When the different races were compared, Indians had the highest aneuploidy rate with 30% for monosomy as well as polysomy. However, the difference was not significant when compared with Malays and Chinese. There were also no significant differences in the incidence of aneuploidy for different TNM stages and Fuhrman grades.

Survival analysis

A total of 41 patients were analyzed for recurrence free survival and overall survival as two cases were lost to follow-up after initial presentation. There were two patients with metastatic recurrence and six deaths from RCC. Survival analysis illustrated no correlation between chromosome 7 aberrations with recurrence and life-span of the examined patients [Figure 2]. Out of those who died from RCC, 1 was monosomy, 4 was diploidy and 1 was polysomy. The mean months for overall survival for diploidy (normal) was 75.22 ± 5.25, 70.86 ± 5.69 for monosomy and 67.83 ± 12.02 for polysomy 7 expression ($\chi^2 = 0.243, P = 0.886$). However, there was a significant link between race and overall survival ($\chi^2 = 6.521, P = 0.038$) as shown in [Figure 3]. The Malays and Indians had a better prognosis compared with the Chinese as none of them died from RCC.
Figure 3: Overall survival by race for patients diagnosed with clear cell renal cell carcinoma and papillary renal cell carcinoma ($P = 0.038$)

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In addition, when stage was considered as a factor on its own for overall survival, it was highly significant ($\chi^2 = 10.87, P = 0.004$) as illustrated in [Figure 4]. As the stage of the cancer increased the survival of the patient decreased. In this study, grade when considered a factor on its own was not significant ($P = 0.127$) for overall survival. However, for recurrence free survival, there was a near significance ($P = 0.067$) association with tumor grading. The mean months for recurrence free survival for patients with tumor Grades I and II was $76.19 \pm 3.12$ while Grades III and IV was $67.91 \pm 7.43$.

Figure 4: Overall survival by stage at diagnosis for patients with clear cell renal cell carcinoma and papillary renal cell carcinoma ($P = 0.004$)

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Discussion

Diagnosis of RCC is primarily based on the histomorphological features in routine histopathology evaluation. Besides discerning the various RCC subtypes, genetic anomalies can be detected through histopathological assessment. Conventionally, genetic abnormalities in ccRCC have been associated with the deletion of 3p. The mutation of the von-Hippel-Lindau gene localized on chromosome 3p25-p26 is considered vital for the initiation of ccRCC and is inactivated in most sporadic cases of this disease. \cite{13,14} In pRCC, the genetic anomaly is often associated with mutation and duplication of mesenchymal-epithelial transition factor MET proto-oncogene found on chromosome 7. \cite{6} Disregulation of MET is linked to tumourigenesis in pRCC. \cite{15} Chromosomal aberrations in fresh specimens of tumor tissues can be studied with cytogenetic study such as G-band karyotyping, FISH, CISH and most recently, the SISH technique. \cite{12,13,14,15,16} It has been shown that there is an incredibly good concordance between FISH and SISH in assessing human epidermal growth factor receptor 2 status in breast carcinoma. \cite{17} The results in this study demonstrated that SISH is a dependable method in detecting chromosome 7 aberration in ccRCC tissue. The advantages of SISH encompass a shorter (6 h) automated protocol, ability of viewing under conventional bright-field microscope to evaluate the findings as well as allowing permanent storage of slides. \cite{17}

Chromosome 7 polysomy was detected in 1/3 of our pRCC cases. Besides pRCC, chromosome 7 abnormality has also been reported in ccRCC. \cite{8,10,12} This study noted that there was chromosome 7 polysomy in 12.5% of the ccRCC cases, which is between the proportions reported by An et al. and Klatte et al. at 9.5% and 36%, respectively. \cite{9,12} Monosomy was found in 22.5% of our ccRCC cases, but An et al. found none in their case series. \cite{12} Besides the findings by An et al., to our knowledge, no other literature has looked specifically at
chromosome 7 monosomy in RCC subtypes. Based on the observations, chromosome 7 aberrations alone might not be a suitable cytogenetic feature for distinguishing the different RCC subtypes.

Ethnical differences may play a role in the cytogenetics of RCC as there was a higher proportion of Indians with chromosome 7 aneuploidy compared with Chinese and Malays. However, this did not reach statistical significance. Interestingly, there was a significant association between gender and chromosome 7 copy numbers. Polysomy 7 was found in 20.7% of males, but none in females while compared to males, there was a higher proportion of monosomy 7 in female RCC tumors. The implication of this tendency for loss of chromosome 7 in females and gain in males is unknown, but unlikely to have any prognostic value in RCC. This is because in our case series, chromosome 7 ploidy was not significantly associated with the grade or stage of RCC tumor.

Amare Kadam et al. (2001) were the first to show that the higher degree of polysomy 7 was associated with the less positive prognostic factors such as higher tumor grade and stage. Their analysis was based on polysomy 7 dominant and major, with the latter having a higher percentage of tumor cells with chromosome 7 gain. There was no significant association of overall polysomy 7 with tumor grade, but polysomy 7 major had significantly higher tumor grade compared to polysomy 7 dominant. The reason for the difference between Amare Kadam et al. and our polysomy 7 prognosis findings could be due to the different analysis method used. There could also be variations in cytogenetics between different ethnic groups as our case series consisted of Malays, Chinese and Indians.

Further evaluation like clinical follow-up is important in survival prognosis. This study assessed the survival and recurrence rate with regards to chromosome 7 aberration in RCC. We could not establish any significant association between chromosome 7 anomalies and recurrence or survival of patients. However, the Chinese seem to have a worse survival prognosis compared with the other two races. Similarly, a study by Singam et al. (2010) illustrated that the Chinese population had significantly advanced Stage (III and IV) cancers at diagnosis compared with Malays and Indians. The etiology behind this occurrence is still considered a new phenomenon. These differences are also seen among ethnic groups from the western nations; the black populations at younger median age present significantly higher localized cancers than the whites.

There was also a significant association between staging and overall survival. However, grade did not reach statistical significance for survival in our RCC cases. For this reason, staging and grading systems that combine the pathological features with additional prognostic variables such as the "University of California Los Angeles Integrated (UCLA) Staging System" may be superior compared with any one predictor alone.

**Conclusion**

SISH technique may be an alternative method to detect chromosome anomalies in paraffin-embedded tissue sections. Although chromosome 7 aberrations were noted in RCCs in our case series, there was no co-relation between the chromosome 7 anomalies with nuclear grading, staging or survival. Stage was significantly associated with survival while ethnicity may influence the cytogenetics and prognosis of RCC.

**References**


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Figures

[Figure 1], [Figure 2], [Figure 3], [Figure 4]

Tables

[Table 1]