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Ploidy changes between diagnosis and relapse in childhood renal tumours

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Summary. Ploidy patterns, analysed by flow cytometry (FCM) and image analysis (IA), were investigated at relapse in a group of six children with renal tumours [five Wilms' tumour (WT) and one bone metastasizing renal tumour of childhood (BMRTC)] and results compared with diagnostic profiles. IA detected one or more aneuploid populations in five of 12 tumours which were diploid on FCM. Patterns in three of six patients [two with unfavourable histology (UH) and one with favourable histology (FH)] were aneuploid at diagnosis and relapse, two patients (one FH, one BMRTC) developed aneuploid features at relapse and one patient with a tetraploid tumour was diploid at relapse. Histology patterns were similar at diagnosis and relapse in all patients. Three of six patients (two UH, one BMRTC) have died of disease. This report highlights (1) the superiority of IA over FCM in detecting aneuploid populations and (2) changes in ploidy status which have not previously been reported in these tumours. Overinterpretation of DNA status at relapse may prove misleading.

Key words: Bone metastasizing renal tumour of childhood (BMRTC) – Ploidy changes – Relapse – Wilms' tumour

We have previously described an association between DNA status at diagnosis and prognosis in a group of patients with Wilms' tumour (WT) who presented to a single institution over a 10-year period [3]. The aim of this study was to compare ploidy patterns in relapsed renal tumours of childhood with diagnostic profiles and to correlate this with clinical behaviour.

Materials and methods

Tissue from recurrent WT was available for analysis in patients 1, 2 and 3 (Table 1) whose diagnostic material was reported in the original study [3]; a 10-month-old female infant who presented with a bone metastasizing renal tumour of childhood (BMRTC; clear cell sarcoma) during the initial study period but was not previously reported, and a further two patients with WT who presented after the initial study completed the study group.

All patients received chemotherapy at diagnosis as per United Kingdom Children's Cancer Study Group (UKCCSG) protocols [6, and unpublished]. Patients 1, 3 and 6 were receiving chemotherapy at the time of relapse and patients 2, 4 and 5 had been off treatment 1 year, 4 months and 5 months respectively; no patient had received radiotherapy to the site of relapse. All diagnostic and relapse material was reviewed by a UKCCSG histopathologist (A.K. or Prof. H.B. Marsden).

Ploidy was analysed by flow cytometry (FCM) using a FACScan (Becton Dickinson) and image analysis (IA) performed using a CAS 100 or 200 Image Analyser (Becton Dickinson), employing a method previously described [3]. Aneuploidy was defined as a DNA index between 1.15 and 1.8 or greater than 2.2, and tetraploidy as more than 10% of cells in G_2M phase of the cell cycle.

Results

Three patients had favourable histology (FH), two had unfavourable histology (UH) which was diffuse anaplastic and one patient had a BMRTC. Histology patterns in all patients did not alter significantly between diagnosis and relapse (Table 1). All patients with UH developed extensive intra-abdominal disease and died. IA detected one or more aneuploid populations in five of 12 tumours which had been diploid on FCM; these tumours are henceforth referred to as aneuploid. Three of six patients with aneuploid tumours at diagnosis had similar patterns at relapse, two patients with diploid tumours (one with BMRTC) at diagnosis developed aneuploid profiles at relapse and one patient with a tetraploid tumour had diploid features at relapse.

Discussion

Although ploidy in childhood renal tumours has also been investigated by a number of other groups [1, 4, 7], there is little information on patterns at relapse. Van Leeuwen et al. [5] reported similar patterns at diagnosis

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Table 1. Clinicopathological details of patients with relapsed renal tumours of childhood

Patient no.	Diagnosis					Relapse					Follow-up[a]
	Age (years)	Stage	Histology	FCM	IA	Site	Interval from diagnosis (months)	Histology	FCM	IA	
1	4.7	III	UH, anaplastic	1.47	1.44	Diffuse intra-abdominal	5	UH, anaplastic	1.0	1.27, 1.53	Died 9 months
2	6.2	IV	UH, anaplastic	1.0	1.56, 1.70	Diffuse intra-abdominal	25	UH, anaplastic	1.89	1.22, 1.42 1.58, 1.73	Died 10 months
3	2.1	II	FH	1.0	1.0	Local	4	FH	1.35	1.70	A/W 89 months
4	0.8	III	BMRTC	1.0	1.1	Diffuse intra-abdominal	16	BMRTC	1.0	1.66	Died 4 days
5	7.3	II	FH	1.0 (16% SG_2M)	1.0 (13% SG_2M)	Isolated lung	11	FH	1.0	1.0	A/W 15 months
6	10.1	III	FH	1.0	1.28	Isolated lung	9	FH	1.04	1.70	A/W 11 months

FCM, Flow cytometry; *IA*, image analysis; *UH*, unfavourable histology; *FH*, favourable histology; *BMRTC*, bone metastasizing renal tumour of childhood; *A/W*, alive and well

[a] From time of relapse

and relapse in their group of four patients, when the criteria used in this study for defining aneuploidy are applied. Similarly, Taylor et al. [8], in a study of neuroblastoma patients, noted consistency of ploidy at diagnosis and relapse.

While the persistence of aneuploid features at relapse was observed in both anaplastic tumours, the transition from a diploid to aneuploid profile in the patient with clear cell sarcoma at relapse, without a significant change in histology, was unexpected and previously unreported. Although electron microscopy (EM) was not performed in this case, others have noted more abundant organelles on EM in this type of tumour at relapse [2]. It is conceivable that extranuclear DNA from such organelles may have been detected by propidium iodide staining. The relevance of such organelles in relation to histogenesis and biological behaviour of such tumours remains to be elucidated.

It is unlikely that chemotherapy was directly responsible for the changes observed at relapse as only one of the patients whose ploidy changed between diagnosis and relapse was on chemotherapy at the time of relapse; similarly, radiotherapy cannot be directly implicated as the site of relapse had not been included in radiotherapy fields. The emergence of an aneuploid pattern at relapse would not appear to confer aggressive behaviour per se on a tumour, as evidenced by the prolonged disease-free survival in patient 3 of this series. This is in contrast to the adverse prognosis documented in those patients with aneuploid tumours at diagnosis [1, 3, 4].

The ability to perform DNA analysis in WT will become more difficult in future trials as the treatment recommended for all stages of disease in most current WT protocols (SIOP and UKCCSG) entails pre-operative chemotherapy following just Trucut biopsies. Because of the small size of tissue samples thus obtained, ploidy assessment will only be possible using IA, which is not widely available. Preference for IA over FCM is also substantiated by the fact that five of 12 tumour samples in this study had aneuploid features on IA which were not detected by FCM. In addition, one could expect that subsequent samples obtained from delayed primary resection will not necessarily correlate with pretreatment samples; this impression is based on histological changes observed after therapy in WT [9]. We will therefore be deprived of the ability to investigate the relevance of ploidy in understanding the natural history of this disease – a point which is particularly relevant in those patients with tumours with favourable histology and no apparent adverse prognostic features at diagnosis who develop recurrent disease.

In conclusion, this study has demonstrated that ploidy status in renal tumours of childhood may be altered between diagnosis and relapse but that this does not necessarily affect prognosis. Overinterpretation of DNA status at relapse, and presumably even after therapy, may prove misleading. The emergence of an aneuploid population in one patient with BMRTC at relapse warrants similar analysis of further such patients.

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