cDNA microarray analysis assists in diagnosis of malignant intrarenal pheochromocytoma originally masquerading as a renal cell carcinoma

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MATERIALS AND METHODS

Intrarenal pheochromocytoma (paraganglioma) is a very rare tumour. Its diagnosis is often difficult to establish because of its rarity and its histological similarity to renal cell carcinoma (RCC). Recently, we examined the molecular signatures of different subtypes of kidney tumours by using cDNA microarray. The signature pattern for one tumour, which was originally diagnosed as granular cell RCC, was clearly distinct from that of any other subtype of kidney tumour, and led us to re-evaluate the case. Haematoxylin and eosin staining revealed histological features suggestive of pheochromocytoma, and immunohistochemical studies showed positive staining for neuroendocrine markers but not for keratin. A germline missense mutation, D119E, in the familial paraganglioma related gene succinate dehydrogenase subunit D (SDHD), was subsequently identified. The treatment modality was revised and radiotherapy was given, to which the patient responded, leading to a reduction in tumour size of 25% within the first month. To our knowledge, this is the first report of an intrarenal pheochromocytoma that was diagnosed with the assistance of cDNA microarray analysis.

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Pheochromocytomas are tumours of neuroectodermal origin arising from chromaffin cells, which produce catecholamines. They are usually derived from the adrenal medulla, but approximately 10% arise from sympathetic ganglia (extra-adrenal pheochromocytomas or paragangliomas). Pheochromocytomas have been described in several well known hereditary cancer syndromes including multiple endocrine neoplasia type 2, von Hippel-Lindau disease, and neurofibromatosis type 1. Hereditary paragangliomas (PGL) have also been associated with germline mutations of the genes that encode three of the four subunits of mitochondrial complex II, succinate dehydrogenase (SDH) B, C, and D. When such tumours occur in extra-adrenal sites, their diagnosis can be challenging; for example, to date there are only three reported cases of intrarenal pheochromocytoma.3,4

We conducted a study of gene expression profiles of kidney tumours using a cDNA microarray technique. These gene expression profiles can serve as the molecular signatures of particular tumours, and different groups of genes may correlate with the behaviour of the tumours (for example, invasiveness, angiogenesis), clinical outcome, and drug response.5 In this report, we describe how, with the help of gene expression profiling, immunohistochemical staining, and mutation analysis, we identified a case of intrarenal pheochromocytoma that originally masqueraded as a renal cell carcinoma.

cDNA microarray findings

In 2001, we were analysing the gene expression profiles of seven different histological subtypes of kidney tumours to determine if these subgroups can be distinguished by their gene expression profiles using microarrays containing 19 968 cDNAs.6 In total, 70 kidney tumours (including 39 clear cell RCCs, one metastatic and seven primary papillary RCCs, six

Abbreviations: AML, acute myeloid leukaemia; MIBG, metaiodobenzylguanidine; RCC, renal cell carcinoma; SDH, succinate dehydrogenase
granular cell RCCs (including the present case), five chromophobe RCCs, two sarcomatoid RCCs, two oncocyto-
mas, three transitional cell carcinomas (TCCs) of the renal pelvis, and five Wilms tumours) were compared with
noncancerous kidney tissues. Based on the expression
patterns of 3560 selected cDNAs, we found distinct molecular
signatures in the clear cell, papillary, chromophobe RCC/
oncocyteoma, TCC, and Wilms subtypes, whereas the six cases
with granular cell RCC did not show a common signature
(fig 3).

**Pathological and immunohistochemical evaluation of “granular cell RCC”**

These “granular RCC” cases were further reviewed histologically by a urological pathologist (XJY), blinded to the study.
All granular cell RCC cases except the present case were
reclassified as clear cell RCC (n = 4) or oncocytoma (n = 1),
which were compatible with gene expression classification.
The present case, which was not clustered with any subtype
of RCC, was also reevaluated using NSE, HMB45, synapto-
physin, S100, and keratin immunohistochemistry (performed as
previously described).7

**Mutation analysis of succinate dehydrogenase genes**
The genes encoding the mitochondrial complex II subunits
SDHA, SDHB, SDHC, and SDHD, were screened for mutations
as previously reported.4 PCR amplification was carried out in
1× PCR buffer (Qiagen) containing 200 μmol/l deoxyribo-
nucleotide triphosphate, 0.6 μmol/l of each primer, 2.5 U Taq
polymerase (Qiagen), and 100–200 ng of tumour DNA
template in a 50 μl volume. PCR conditions were one cycle
of 15 minutes at 95°C then 35 cycles of 1 minute at 95°C,
1 minute at 58°C, and 1 minute at 72°C, which was followed
by one cycle of 10 minutes at 72°C.

PCR amplicons were purified by gel electrophoresis (Bio-
Rad Laboratories, Hercules, CA) and column purification
(Wizard PCR Prep; Promega, Madison, WI), and subjected to
semiautomated sequencing using the aforementioned pri-
mers with a dye terminator method, and sequenced on an
ABI377xl or PE3700 sequencer. The sequencing was repeated
on DNA of the matched normal kidney.

**RESULTS**

Histology showed clusters of tumour cells with granular
cytoplasm and prominent vascularity, suggestive of pheo-
chromocytoma, extensively infiltrating the kidney parench-
yma (fig 2A, B). Further immunohistochemical analysis
showed positive staining for NSE, HMB45, and synapto-
physin (focally positive) and negative staining for keratin in
cancer cells, and positive staining for S100 in sustentacular
cells (fig 2–E). These findings further support the diagnosis
of pheochromocytoma or paraganglioma. Interestingly, we
also identified a germline mutation, D119E, in the
SDHD gene (fig 4). Two common polymorphisms were also found, T891C
in SDHA and C18A in SDHB.

Five years after the nephrectomy, the patient is alive with
continuing disease. He had an enlarging para-aortic mass
without hypertension or elevation of any catecholamine
related markers in blood and urine. Strong adhesion around
the duodenum and inferior vena cava prevented further
surgical resection of the residual tumour. After a diagnosis of

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Figure 1  Imaging results. (A) Abdominal computerised tomography
revealed a solid mass in the right renal area, approximately 9×5 cm in
size, which contained a low density area indicating necrosis. Marked
lymphadenopathy can be seen. (B) Selective renal arteriography with
tumour staining revealed a hypervascular tumour in the middle to lower
pole of the right kidney and a dilated right renal vein.

Figure 2 Intrarenal
pheochromocytoma. (A) Low
magnification section showing tumour
cells (right side) infiltrating renal cortex
(left low corner) (haematoxylin and
eosin). (B) High magnification section
showing tumour cells with fine granular
cytoplasm and rich microvessels
(haematoxylin and eosin). (C) Positively
stained tumour cells (NSE
immunohistochemistry). (D)
Nonreactivity in tumour cells but
reactivity in residual renal tubules
( keratin immunohistochemistry; AE1/
AE3). (E) Positively stained
sustentacular cells ( S-100
immunohistochemistry).
malignant intrarenal pheochromocytoma was established, the patient underwent external beam radiation therapy, delivering 50 Gy to the residual para-aortic tumours. One month after radiation therapy, MRI revealed a 25% reduction in the para-aortic masses.

**DISCUSSION**

Previously, in a microarray study of acute leukaemia, one particular case showed a gene expression profile that fitted neither acute myeloid leukaemia (AML) nor acute lymphoblastic leukaemia. This brought into question the diagnosis of that case as AML. Subsequent analysis found that the tumour had high expression of certain particular genes suggesting a mesenchymal origin. Further cytogenetic study, which revealed a pathognomonic translocation, confirmed it as an alveolar rhabdomyosarcoma, and the treatment for the particular case showed a gene expression profile that fitted neither acute myeloid leukaemia (AML) nor acute lymphoblastic leukaemia. 9 This brought into question the diagnosis of that case as AML. Subsequent analysis found that the tumour had high expression of certain particular genes suggesting a mesenchymal origin. Further cytogenetic study, which revealed a pathognomonic translocation, confirmed it as an alveolar rhabdomyosarcoma, and the treatment for the patient was revised accordingly.

Similarly, our microarray based molecular classification study of RCC revealed that a previous diagnosis of RCC was actually a rare malignant intrarenal pheochromocytoma. This change in diagnosis was only possible because of the gene expression microarray. The molecular profile of this case clearly did not fit with any of the other RCC cases, leading to further investigation by immunohistochemical staining and mutation analysis. Using normal kidney tissues as a control allowed us to find a marked difference in the molecular signature of this tumour. Ideally, we should have obtained the tumour's own molecular signature compared to normal adrenal tissues, but we did not have enough RNA for obtaining particularly altered gene expressions. In addition, we found that this patient carries a germline missense mutation, D119E, in exon 4 of SDHD. This amino acid, and those surrounding it, is highly conserved down to mouse and rat. The D119E mutation is predicted to disrupt assembly of complex II. The patient does not have a family history of hereditary paraganglioma and the mutation could well represent a de novo mutation. Unfortunately, neither parent was available for mutation analysis.

Because of further immunohistochemical and molecular studies, the treatment modality for this patient was revised, a change to which the patient has responded positively. The diagnosis of malignant pheochromocytoma (paraganglioma) instead of RCC had a great effect on the treatment approach. Interferon and interleukin-2 are still the mainstay treatment for metastatic RCC, and radiation therapy is generally considered ineffective except for palliation in bone metastasis or stereotactic radiosurgery for brain metastasis. In contrast, there are several reports on the response of malignant pheochromocytoma to radiation therapy, including external beam radiotherapy or radiolabelled metaiodobenzylguanidine (MIBG). As MIBG scintigraphy revealed no accumulation of MIBG in the tumour, radiolabelled MIBG therapy was not indicated in this case. Combination chemotherapy of cyclophosphamide, vincristine, and dacarbazine or of these three agents with doxorubicin have also been reported as effective in treating malignant pheochromocytoma. After providing these treatment options to the patient, he decided to undergo external beam radiotherapy for his para-aortic tumours. He has experienced a partial response (25% reduction in size to the radiotherapy), and continues under follow up.

**CONCLUSION**

We report a very rare case of intrarenal pheochromocytoma in a 26 year old man whose final diagnosis was first suspected because of microarray gene expression profiling. This then led to further immunohistochemical staining for neuroendocrine markers and mutation analysis of the known paraganglioma associated genes, which revealed an unexpected predisposing germline mutation. This case illustrates the importance and clinical implications of microarray expression profiling.

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**Figure 3** Clustering of 70 kidney tumours. The clustering of patients (using Pearson’s correlation) is based on global gene expression profiles. The tumours clustered into two broad groups with one group consisting of primarily clear cell RCC and the other consisting of all other kidney tumours. Five chromophobe RCCs and two oncocytomas are clustered close together. Eight papillary RCCs, five Wilms tumours, and three TCC are clustered together as groups. The case “granular 5,” which has its own distinct molecular signature, does not cluster with any other type of kidney tumour.

**Figure 4** Germline D119E (exon 4) mutation; the succinate dehydrogenase subunit D (SDHD) gene.

GAT -> GAG
Asp -> Glu
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