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MAPPING OF SUCCINATE DEHYDROGENASE LOSSES IN 2258 EPITHELIAL NEOPLASMS

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Abstract

Losses in the succinate dehydrogenase (SDH) complex characterize 20–30% of extra-adrenal paragangliomas and 7–8% of gastric GISTs, and rare renal cell carcinomas. This loss is reflected as lack of the normally ubiquitous immunohistochemical expression of the SDH subunit B (SDHB). In paragangliomas, SDHB loss correlates with homozygous loss of any of the SDH subunits, typically by loss-of-function mutations. The occurrence of SDHB losses in other epithelial malignancies is unknown. In this study, we immunohistochemically examined 2258 epithelial, mostly malignant neoplasms including common carcinomas of all sites. Among renal cell carcinomas, SDHB loss was observed in 4 of 711 cases (0.6%) including a patient with an SDHB-deficient GIST. Histologically the SDHB-negative renal carcinomas varied. There was one clear cell carcinoma with a high nuclear grade, one papillary carcinoma type 2, one unclassified carcinoma with a glandular pattern, and one oncocytoid low-grade carcinoma as previously described for SDHB-negative renal carcinoma. None of these patients was known to have paragangliomas or had loss of SDHA expression in the tumor. Three of these patients had metastases at presentation (2 in the adrenal, one in the retroperitoneal lymph nodes). There were no cases with SDHB-loss among 64 renal oncocytomas. SDHB-losses were not seen in other carcinomas, except in one prostatic adenocarcinoma (1/57), one lymphoepithelial carcinoma of the stomach, and one (1/40) seminoma. Based on this study, SDHB-losses occur in 0.6% of renal cell carcinomas and extremely rarely in other carcinomas. Some of these renal carcinomas may be clinically aggressive. The clinical significance and molecular genetics of these SDHB-negative tumors requires further study.

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Keywords

succinate dehydrogenase subunit B; SDHB; renal cell carcinoma; prostatic carcinoma; gastric lymphoepithelial carcinoma

INTRODUCTION

Succinate dehydrogenase is a key heterotetrameric enzyme complex of the energy metabolism located in the mitochondrial inner membrane and involved in the Krebs cycle and oxidative phosphorylation. [1] Loss of this complex is a known event and oncogenic mechanism up to 30% of extra-adrenal paragangliomas, and this loss is generally associated with a germline loss-of-function mutation in one of the SDH-subunit proteins, most commonly SDHB or SDHD, and rarely SDHC, or SDHA. The loss seems to be compounded by somatic inactivation of the other copy of the mutated subunit gene leading to total loss of that subunit protein and dissolution of the complex. Immunohistochemically observed lack of SDHB expression is a practical marker of the functional deficiency of the SDH-complex, and this loss has also been considered an indirect marker of an SDH-subunit germline mutation, at least in paragangliomas. [2–6]

Similar losses in the SDH-complex occur in 7–8% of gastric GISTs, especially those occurring in young patients. Loss of the SDH-complex is a known pathogenetic event in GIST and is also associated with SDH-subunit germline mutations. [7–11] Loss of SDH-complex function activates pseudohypoxia signaling via HIF1/HIF2-alpha and leads to dysregulation of cellular proliferation and adhesion rendering the cell a neoplastic phenotype. [12–15] In GIST, it is additionally known to activate oncogenic insulin-like growth factor 1 receptor signaling. 8,[16]

In carcinomas, the loss of SDHB was initially detected in an early onset renal cell carcinoma [17] and subsequently in SDHB-mutation syndrome-associated renal carcinomas, which seem to have distinctive oncocytoid morphology with cytoplasmic pseudoinclusions. [18–20] Few reports exist on other types of SDHB-negative renal cell carcinomas. [21–23] However, the frequency of this event is unknown. Loss of the SDH complex in the other malignant epithelial neoplasms has not been explored. In this study we systematically examined 711 renal and 1537 non-renal carcinomas for SDHB loss.

MATERIALS AND METHODS

Approximately 2200 carcinomas and other extensively documented epithelial neoplasms (mostly carcinomas) were arranged in multitumor blocks containing 30–50 tumors per block as previously described. [24] A cohort of renal carcinomas from patients <40 years of age was available in a tissue microarray format. Tumors originated from Northern and Central Europe, and from the United States.

Immunohistochemical studies were performed with a Leica BondMax automated stainer using the BondMax detection kit. Primary antibody to SDHB 21A11 (ABCAM, Cambridge, Massachusetts) was used in a dilution of 1:1000 and incubated for 30 min. Diaminobenzidine was used as the chromogen, followed by a light hematoxylin counterstain. SDHB-negative cases were also studied for SDHA expression (primary antibody 5A11, ABCAM, 1:1000) using a similar methodology.

Succinate dehydrogenase subunit B (SDHB) loss was considered present when tumor cells lacked granular cytoplasmic staining showing a contrast with positive non-neoplastic

adjacent elements (endothelial, epithelial, lymphoid or myoid cells) with granular immunostaining.

RESULTS

Most carcinomas and other epithelial tumors expressed succinate dehydrogenase subunit B (SDHB), as detected by immunohistochemistry. Practically all tumor cells showed granular cytoplasmic positivity, reflecting mitochondrial expression of SDHB. Positivity was also observed in various non-neoplastic elements, such as vascular endothelia, fibroblasts, lymphoid cells, and epithelia.

Renal tumors

Results on renal epithelial tumors are summarized in Table 1 and illustrated in Fig. 1. The overall frequency of SDHB-negative renal carcinomas was 4 of 711 (0.6%). These carcinomas included 1 clear cell carcinoma with high nuclear grade (grade 3), metastatic to the adrenal in a 40-year-old male (Fig. 1A). Case 2 was a retroperitoneal nodal metastasis of a papillary type 2 renal carcinoma in a 35-year-old male. This tumor featured papillary frond formation, large nuclei, and high-grade nuclear atypia (Fig. 1B).

The third tumor, renal carcinoma of an unclassified type in a 44-year-old male, had oncocytoid morphology but glandular architecture (Fig. 2 C). This tumor also had a concurrent adrenal metastasis. This patient's mother had an early onset renal carcinoma diagnosed at age of approximately 25 years. Unfortunately, that tumor was not available for review.

The fourth tumor (a 7 cm upper pole right kidney mass) had oncocytoid cytology and was composed of epithelioid, eosinophilic cells with focal cytoplasmic clearance and pale cytoplasmic inclusions (Fig. 2D). This patient was a 59-old-woman who had an SDH-deficient GIST 40 years earlier and developed liver metastasis of her gastric GIST two years after the diagnosis of renal carcinoma (42 years after the primary gastric GIST).

None of the 64 renal oncocytomas or 47 renal tumors from a microarray cohort of patients <40 years of age had an SDHB loss. None of the SDHB-negative renal carcinomas was SDHA-negative or was known to be associated with paragangliomas. Unfortunately, material was insufficient for SDH-subunit mutation analysis.

Non-renal epithelial tumors

In other carcinomas, loss of SDHB was detected in one (1/57) prostatic adenocarcinoma of Gleason grade 4+4 (Fig. 2A), and a gastric, EBER-positive lymphoepithelial carcinoma (Fig. 2B). However, none of the 10 nasopharyngeal lymphoepitheliomas or their metastases was SDHB-negative. None of the other carcinomas or malignant mesotheliomas showed an SDHB-loss.

In addition 1 of 40 testicular seminomas had a SDHB-loss (Fig. 2C) but none of the non-seminomatous germ cell tumors had this loss. All SDHB-negative tumors studied here were positive for SDHA and there was no known association with paraganglioma. Computerized searches in the participating institutions also failed to identify more patients with seminoma and paraganglioma.

Exceptions to SDHB-positivity of common carcinomas also included focal SDHB-loss in the keratinizing component of well-differentiated squamous cell carcinomas. However, this was considered related to low mitochondrial content and low viability of these components

DISCUSSION

Losses in the succinate dehydrogenase complex are well known for 30% of extra-adrenal paragangliomas[1–6], 7–8% of gastric gastrointestintestinal stromal tumors (especially in young patients) [7–11], and rare renal carcinomas.[17–23] In paragangliomas, the loss in generally associated with loss-of-function mutations in one of the subunit of the succinate dehydrogenase complex 4.

In this study, we examined loss of succinate dehydrogenase subunit B (SDHB) expression in 711 renal carcinomas and 1537 other epithelial neoplasms, including the common carcinomas, mesotheliomas and germ cell tumors.

Four of 711 renal carcinomas showed SDHB loss (0.6%). These tumors had a heterogenous morphology, including 1 clear cell carcinoma or high nuclear grade, one papillary carcinoma type 2 with high-grade cytology, and two carcinomas with oncocytoid features: one glandular and another solid. Three of these 4 patients had metastases, indicating that these tumors can be clinically aggressive. Frequent metastases were also reported in a recent clinical series of SDHB-negative renal carcinomas of SDHB, SDHC, and SDHD mutation carriers. [25]

This heterogeneous morphology indicates that the SDHB-negative renal carcinomas cannot be identified by histologic appearance alone. Neither can they be identified by patient age alone, as they seem to represent a low percentage of renal carcinomas of patients < 40 years, as no cases were found in a tumor array of such a special subcohort.

The number of reported SDHB-negative renal cell carcinomas remains small and also indicates morphologic heterogeneity. Notably, papillary renal carcinoma type 2 observed here among the SDHB-negative renal carcinomas has been reported among SDHB-negative renal carcinomas.[21] However, this histologic subtype has been previously also been associated with hereditary leiomyomatosis and kidney cancer (HLRCC) syndrome, which is caused by fumarate hydratase germline mutation. [26]

SDHB-negative renal carcinomas occurring in SDHB mutation syndromes seem to have distinctive oncocytoid morphology with cytoplasmic inclusions. [18–20,23] One such tumor was included in this study; a tumor in a patient with a SDH-deficient GIST. As this case showed, different tumors can be highly asynchronous in these patients making it more difficult to diagnose the multiple tumor syndromes. Because none of the 64 renal oncocytomas tested here showed an SDHB-loss, typical oncocytoma does not seem associated with an SDHB-loss.

Clear cell renal cell carcinoma morphology may also be associated with SDH-loss. One high-grade clear cell carcinoma was SDHB-negative in this study, and a previous report details occurrence of a low-grade renal clear cell carcinoma associated with SDHC-germ line mutation syndrome. The same patient subsequently had a papillary type 1 renal carcinoma in the kidney, but this tumor was SDHB-positive, despite showing allelic losses in SDHC, similar to the SDHC-negative clear cell carcinoma. [22] A recent clinical series also concluded that SDHC germ line mutation syndrome may be associated with classic clear cell carcinoma. [25]

Loss of SDHB expression is very rare in non-renal carcinomas and was never observed in malignant mesothelioma, or carcinomas of breast, lung, colon, and pancreas. However, SDHB-loss was detected in one prostatic adenocarcinoma and one lymphoepithelial carcinoma of the stomach in this study. Neither of these patients was known to have paragangliomas or SDH-deficient GISTs, and the significance of these losses and their

relationship to SDH subunit gene mutations requires further study. Lack of SDHB-loss in nasopharyngeal carcinomas does not indicate that this loss would be common in those tumors, although the available sample (10 cases) was small.

One seminoma in this study showed an SDHB loss. Although seminoma is not generally known to be related to SDH deficiency, there are two reports of co-occurrence of paraganglioma and seminoma, one in a human and one in a dolphin. The patient was an SDHD-mutation carrier from a family with paragangliomas having a testicular seminoma with LOH in the SDHD-locus; SDHB-immunostaining was not evaluated in that case. [27] A stranded spotted dolphin was found to have metastatic testicular seminoma, adrenal pheochromocytoma, and a Sertoli cell tumor; there was no data on SDHB immunohistochemistry. [28] Our observations and these reports suggest that seminoma may be an infrequent component of the SDH tumor syndrome, and additional studies are needed to further explore this connection.

Apart of the SDH-loss related to the SDH tumor syndrome, some of the SDHB-losses may be associated with a functional state of terminally differentiated cells, as observed in keratining cells of squamous cell carcinoma. These cells are known to be metabolically less viable, and therefore apparent SDHB-loss most likely only reflects paucity or total of loss of mitochondria.

In conclusion, we report 0.6% frequency of SDHB-negativity in renal cell carcinomas and show heterogeneous morphology for these tumors, including examples with papillary carcinoma type 2, clear cell, and oncocytoid histology. Non-renal carcinomas had SDHB-loss only in isolated examples: one prostatic carcinoma, one lymphoepithelial carcinoma of the stomach, and one seminoma. Additional studies are needed to establish phonotype-genotype correlation and the clinical significance of SDHB-negative renal and non-renal carcinomas.

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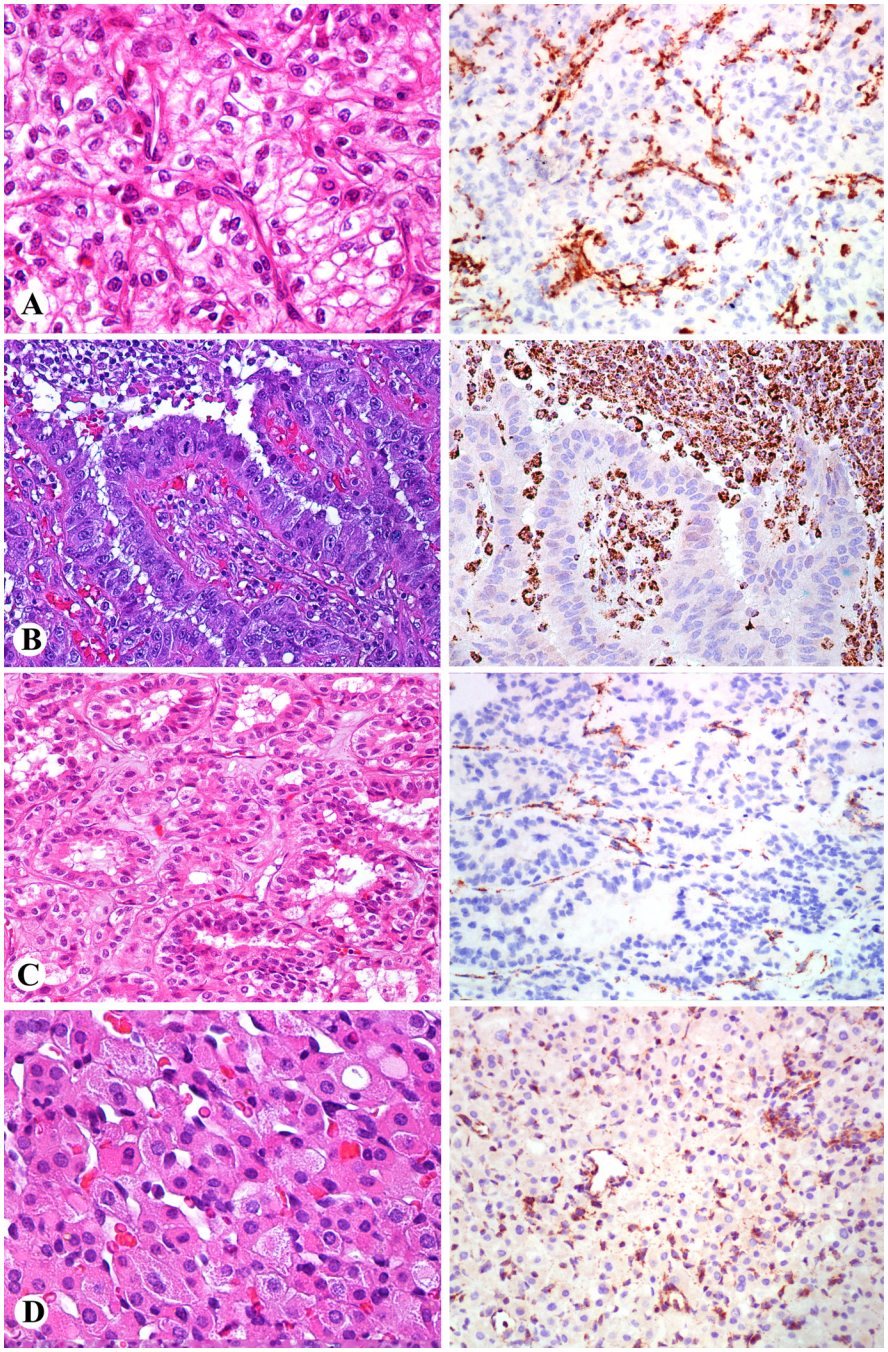

Figure 1.
Paired histologic images and SDHB immunostains of four SDHB-negative renal cell carcinomas. Note that all immunostains show negative tumor cells are positive stromal elements. A. Clear cell carcinoma with high nuclear grade. B. Papillary type 2 carcinoma. C. Oncocytoid carcinoma with a glandular pattern. D. An oncocytoid low-grade carcinoma with pale cytoplasmic inclusions.

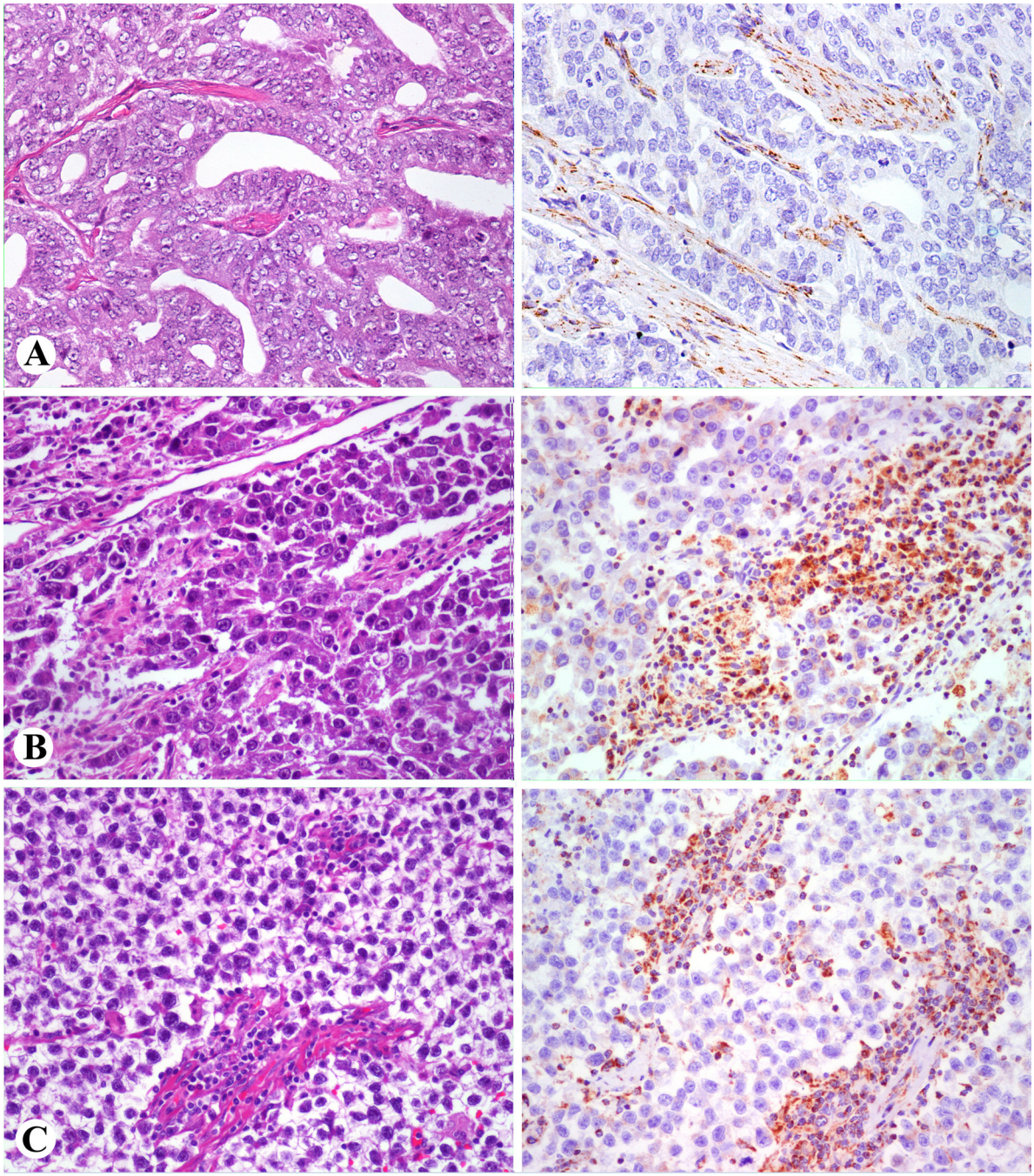

Figure 2.
Paired histologic images and SDHB immunostains of three SDHB-negative non-renal malignant epithelial neoplasms. Note that all tumor cells are negative for SDHB, but stromal elements are positive. A. Prostatic adenocarcinoma (Gleason 4+4). B. Lymphoepithelial carcinoma of the stomach. C. Testicular seminoma.

Table 1

Losses of SDHB in 711 cases of different types of renal carcinomas and other renal epithelial neoplasms. Positive finding denotes a loss of SDHB expression.

Clear cell carcinoma, low-grade	0/352
Clear cell carcinoma, high-grade	1/201
Chromophobe carcinoma	0/24
Papillary carcinoma, type 1	0/25
Papillary carcinoma, type 2	1/8
Sarcomatoid carcinoma	0/14
Unclassified carcinoma	2/16
Onkocytoma of kidney	0/64
Hybrid onkocytoma - chromophobe carcinoma	0/4
Metanephric adenoma	0/2
Mixed epithelial and stromal tumor	0/1
Total	4/711

Table 2

Occurrence of immunohistochemical SDHB losses in 1547 non-renal carcinomas and other epithelial neoplasms. Positive finding denotes loss of SDHB expression.

Adenoid cystic carcinoma	0/31
Adrenocortical carcinoma	0/31
Breast, ductal carcinoma	0/170
Breast, lobular carcinoma	0/47
Breast, mucinous carcinoma	0/11
Cervix, squamous carcinoma	0/22
Colon, adenocarcinoma	0/109
Intestines, undifferentiated carcinoma	0/29
Endometrium, adenocarcinoma	0/54
Esophagus, squamous cell carcinoma	0/22
Malignant mesothelioma	0/80
Larynx, squamous cell carcinoma	0/37
Livr, cholangiocarcinoma	0/18
Liver, hepatocellular carcinoma	0/38
Lung, adenocarcinoma	0/51
Lung, small cell carcinoma	0/38
Lung, squamous cell carcinoma	0/44
Lymphoepithelial carcinoma, nasopharynx	0/10
Ovary, serous carcinoma	0/120
Ovary, endometrioid and clear cell carcinoma	0/35
Pancreas, adenocarcinoma	0/57
Pancreas, neuroendocrine tumor	0/16
Parathyroid, adenoma	0/55
Prostate, adenocarcinoma	1/57
Rectum, adenocarcinoma	0/26
Stomach, adenocarcinoma	1/72
Thymoma	0/62
Thyroid, anaplastic carcinoma	0/10
Thyroid, follicular carcinoma	0/28
Thyroid, papillary carcinoma	0/43
Testis, embryonal carcinoma	0/24
Testis, seminoma	1/40
Urinary bladder, transitional cell carcinoma	0/60
Total	3/1547