ORIGINAL ARTICLE

# Pax-5 immunoexpression in various types of benign and malignant tumours: a high-throughput tissue microarray analysis

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**Background:** Pax-5 belongs to the *Pax* gene family transcription factors that play an important role in organogenesis and in B cell ontogeny. It is expressed in B cell non-Hodgkin's lymphoma (B-NHL), Hodgkin's lymphoma (HL) and neuroendocrine carcinomas. However, its expression in other tumour types is not fully explored.

**Aims and methods:** To determine Pax-5 expression in other tumour types, immunohistochemistry was performed on 3758 benign and malignant tumours using multiple tumour microarrays, as well as on whole sections.

**Results:** Pax-5 was expressed in 108/118 (91.5%) B-NHLs, in 60/70 (85.7%) HLs and in 0/7 T cell lymphomas. In addition, Pax-5 was seen in 24/34 (70.6%) Merkel cell carcinomas, 42/53 (79.2%) small cell carcinomas, 1/164 (0.6%) breast carcinomas, 2/204 (1%) endometrial adenocarcinomas and 1/452 (0.2%) urothelial carcinoma of the bladder.

**Conclusion:** Despite its expression in a small subset of malignancies of epithelial origin, Pax-5 is still a good and reliable immunomarker in diagnosing B-NHL, HL and neuroendocrine carcinomas.

The *pair box* (*PAX*) gene family encodes a family of regulatory proteins that are involved in transcriptional control of organ development and tissue differentiation.[1] At least four isoforms have been identified, of which the Pax-5 isoform has been the most extensively studied. Pax-5 is located at chromosome 9q13 and encodes a nuclear DNA-binding protein in B cells known as B cell-specific activation protein.[2 3] Pax-5 is continuously expressed from the B lymphoid progenitors to the mature B cell stage. Pax-5 has an early function where it is required for commitment of lymphoid progenitors to the B cell pathway and also has an essential function in late B cell stages by maintaining the function, identity and survival of mature B lymphocytes. Nevertheless, studies revealed that Pax-5 is downregulated in the plasma cell stage.[4–6] In addition, Pax-5 has an important role in central nervous system development in mice.[7 8] In humans, the role of Pax-5 in brain development is not yet fully known, but data showed that it is present in the mesencephalon and the spinal cord, and it is lost in the neonatal and adult cerebellum.[9] Besides its role in B cell ontogeny and organogenesis, Pax-5 has a critical role in tumorigenesis.[10] Deregulation of Pax-5 protein was seen in medulloblastomas, neuroblastomas and astrocytomas, and in bladder carcinomas.[11–15] Furthermore, data suggested that re-expression of the Pax-5 gene can increase tumour proliferation and motility, inhibit apoptosis and, subsequently, promote tumour development and progression. However, new evidence in brain tumours from a mouse model suggested that Pax-5 might play a role in tumour progression but not in tumour initiation.[16]

Two studies used Pax-5 monoclonal antibody in formalin-fixed paraffin wax-embedded tissues. The first explored Pax-5 in lymphomas and showed that anti-Pax-5 can be expressed in precursor and mature B cell non-Hodgkin's lymphoma (NHL)/leukaemia and in Hodgkin's lymphoma (HL).[17] The second study investigated Pax-5 in neuroendocrine carcinomas and found Pax-5 reactivity in small cell carcinomas (SCCs) and Merkel cell carcinomas (MCCs).[18] However, can other tumour types express Pax-5? If yes, in what percentage? The answers are still unknown, and our study will be the first to address these important questions. On the basis of any pathologist's experience and as in other immunomarkers before Pax-5, there is a probability that some tumours will express Pax-5 and this knowledge will be crucial to avoid pitfalls and misdiagnosis. To accomplish this goal, a multiple tumour microarray technique has been used. The multiple tumour microarray technique is a high-throughput technique that has been proven by numerous previous studies to be efficient, time saving and very practical for evaluations of the expression of immunohistochemical markers.[19–21]

## MATERIALS AND METHODS

### Tissue microarray and immunohistochemistry

A formalin-fixed, paraffin wax-embedded tissue for multiple tumour tissue microarray construction was used. The microarray was constructed as described previously.[19 22] In addition, whole sections from 28 cases of MCCs, 40 SCCs of the lung and 3 desmoplastic small round cell tumours were included in the study. An H&E-stained section was evaluated for the presence of the tumour by light microscopy. Sections (4 μm) were processed for immunohistochemistry (IHC). Endogenous peroxidase was blocked with 0.3% hydrogen peroxidase for 30 min. Antigen retrieval was carried out in a high pH buffer for 30 min in a steamer-cooker. Subsequently, sections were incubated with Pax-5 antibody (clone 24, 1:300, BD Biosciences, New Jersey, USA) at room temperature for 30 min. A biotin-free horseradish peroxidase enzyme-labelled polymer of the Envision plus detection system was added

**Abbreviations:** HL, Hodgkin's lymphoma; IHC, immunohistochemistry; MCC, Merkel cell carcinoma; NHL, non-Hodgkin's lymphoma; PAX, pair box; RT, reverse transcriptase; SCC, small cell carcinoma; UC, urothelial carcinoma

**Table 1** Pax-5 immunoexpression in 3758 benign and malignant tumours

| Organs | Negative | Weakly positive | Moderately positive | Strongly positive | Total |
|---|---|---|---|---|---|
| Haematopoietic neoplasms | | | | | |
| Total | 33 | 32 | 31 | 105 | 201 |
| Total Hodgkin's lymphoma | 10 | 15 | 12 | 33 | 70 |
| Total non-Hodgkin's lymphoma | 10 | 17 | 19 | 72 | 118 |
| MALT lymphoma | 5 | 14 | 3 | 27 | 49 |
| Follicular lymphoma | 3 | 0 | 11 | 18 | 32 |
| CLL | 1 | 0 | 0 | 0 | 1 |
| Burkitt's lymphoma | 0 | 0 | 0 | 3 | 3 |
| Mantel cell lymphona | 0 | 0 | 0 | 1 | 1 |
| Diffuse large B cell lymphoma | 1 | 3 | 5 | 23 | 32 |
| TCL | 7 | 0 | 0 | 0 | 7 |
| AML | 1 | 0 | 0 | 0 | 1 |
| CML | 5 | 0 | 0 | 0 | 5 |
| Neuroendocrine tumours | | | | | |
| Total | 122 | 27 | 19 | 20 | 188 |
| Lung | 9 | 19 | 12 | 8 | 48 |
| Pheochromocytoma | 29 | 0 | 0 | 0 | 29 |
| Paraganglioma | 10 | 0 | 0 | 0 | 10 |
| Carcinoid | 46 | 0 | 0 | 0 | 46 |
| Skin—Merkel cell carcinoma | 10 | 7 | 5 | 12 | 34 |
| Urinary bladder—small cell carcinoma | 2 | 1 | 2 | 0 | 5 |
| PNET | 16 | 0 | 0 | 0 | 16 |
| Squamous cell carcinomas | | | | | |
| Total squamous cell carcinoma | 404 | 0 | 0 | 0 | 404 |
| Head and neck | 120 | 0 | 0 | 0 | 120 |
| Skin | 138 | 0 | 0 | 0 | 138 |
| Skin—undefined source | 46 | 0 | 0 | 0 | 46 |
| Anus | 4 | 0 | 0 | 0 | 4 |
| Vulva | 42 | 0 | 0 | 0 | 42 |
| Penis | 46 | 0 | 0 | 0 | 46 |
| Cervix | 46 | 0 | 0 | 0 | 46 |
| Vagina | 5 | 0 | 0 | 0 | 5 |
| Oesophagus | 38 | 0 | 0 | 0 | 38 |
| Urinary bladder | 8 | 0 | 0 | 0 | 8 |
| Lung | 49 | 0 | 0 | 0 | 49 |
| Adenocarcinomas | | | | | |
| Total adenocarcinoma | 749 | 0 | 3 | 0 | 752 |
| Oesophagus | 8 | 0 | 0 | 0 | 8 |
| Stomach | 67 | 0 | 0 | 0 | 67 |
| Intestinal type | 46 | 0 | 0 | 0 | 46 |
| Diffuse type | 21 | 0 | 0 | 0 | 21 |
| Small intestine | 11 | 0 | 0 | 0 | 11 |
| Colon | 48 | 0 | 0 | 0 | 48 |
| Gall bladder | 30 | 0 | 0 | 0 | 30 |
| Pancreas | 49 | 0 | 0 | 0 | 49 |
| Lung | 49 | 0 | 0 | 0 | 49 |
| Breast | 163 | 0 | 1 | 0 | 164 |
| Lobular | 37 | 0 | 0 | 0 | 37 |
| Ductal | 81 | 0 | 1 | 0 | 82 |
| Mucinous | 26 | 0 | 0 | 0 | 26 |
| Tubular | 19 | 0 | 0 | 0 | 19 |
| Endometrium | 202 | | 2 | | 204 |
| Endometroid type | 181 | 0 | 2 | 0 | 183 |
| Serous type | 21 | 0 | 0 | 0 | 21 |
| Ovary | 127 | 0 | 0 | 0 | 127 |
| Endometroid | 48 | 0 | **0** | 0 | 48 |
| Serous | 65 | 0 | 0 | 0 | 65 |
| Mucinous | 14 | 0 | 0 | 0 | 14 |
| Uterine cervix | 2 | 0 | 0 | 0 | 2 |
| Prostate | 149 | 0 | 0 | 0 | 149 |
| Urinary bladder | 5 | 0 | 0 | 0 | 5 |
| Salivary gland adenocarcinoma | 3 | 0 | 0 | 0 | 3 |
| Germ cell tumours | | | | | |
| Testis | 77 | 0 | 0 | 0 | 77 |
| Seminoma | 16 | 0 | 0 | 0 | 16 |
| Non-seminoma | 61 | 0 | 0 | 0 | 61 |
| Ovarian germ cell | 5 | 0 | 0 | 0 | 5 |
| Malignant soft tissue tumours | | | | | |
| Total | 179 | 0 | 0 | 0 | 179 |
| Leiomyosarcoma | 49 | 0 | 0 | 0 | 49 |
| Kaposi's sarcoma | 30 | 0 | 0 | 0 | 30 |
| Malignant fibrohistiocytoma | 29 | 0 | 0 | 0 | 29 |
| Liposarcoma | 29 | 0 | 0 | 0 | 29 |
| Angiosarcoma | 3 | 0 | 0 | 0 | 3 |
| Monophasic synovial sarcoma | 3 | 0 | 0 | 0 | 3 |

**Table 1** Continued.

| Organs | Negative | Weakly positive | Moderately positive | Strongly positive | Total |
|---|---|---|---|---|---|
| Fibrosarcoma | 6 | 0 | 0 | 0 | 6 |
| Rhabdomyosarcoma | 13 | 0 | 0 | 0 | 13 |
| Malignant Schwannoma | 9 | 0 | 0 | 0 | 9 |
| DFSP | 2 | 0 | 0 | 0 | 2 |
| Endometroid stromal tumour | 4 | 0 | 0 | 0 | 4 |
| Alveolar sarcoma | 1 | 0 | 0 | 0 | 1 |
| Epithelioid sarcoma | 1 | 0 | 0 | 0 | 1 |
| Brain tumours | | | | | |
| Total | 147 | 0 | 0 | 0 | 147 |
| Glioblastoma multiforme | 50 | 0 | 0 | 0 | 50 |
| Astrocytoma | 48 | 0 | 0 | 0 | 48 |
| Oligodendroglioma | 29 | 0 | 0 | 0 | 29 |
| Ependymoma | 12 | 0 | 0 | 0 | 12 |
| Olfactory neuroblastoma | 3 | 0 | 0 | 0 | 3 |
| Medulloblastoma | 5 | 0 | 0 | 0 | 5 |
| Other tumour types | | | | | |
| Total | 9 | 1 | 0 | 0 | 986 |
| Thyroid | 97 | 0 | 0 | 0 | 97 |
| Follicular | 48 | 0 | 0 | 0 | 48 |
| Papillary | 40 | 0 | 0 | 0 | 40 |
| Medullary | 9 | 0 | 0 | 0 | 9 |
| Kidney | 90 | 0 | 0 | 0 | 90 |
| RCC | 56 | 0 | 0 | 0 | 56 |
| Papillary | 29 | 0 | 0 | 0 | 29 |
| Chromophobe | 5 | 0 | 0 | 0 | 5 |
| Salivary gland | 61 | 0 | 0 | 0 | 61 |
| Mucoepidermoid carcinoma | 6 | 0 | 0 | 0 | 6 |
| Adenoid cystic carcinoma | 50 | 0 | 0 | 0 | 50 |
| Acinic cell carcinoma | 7 | 0 | 0 | 0 | 7 |
| Urothelial carcinoma of the bladder | 451 | 1 | 0 | 0 | 452 |
| Hepatocellular carcinoma of liver | 45 | 0 | 0 | 0 | 45 |
| Skin basal cell carcinoma | 48 | 0 | 0 | 0 | 48 |
| Lymphoepithelial carcinoma of pharynx | 5 | 0 | 0 | 0 | 5 |
| Malignant mesothelioma | 25 | 0 | 0 | 0 | 25 |
| Melanoma of skin | 48 | 0 | 0 | 0 | 48 |
| Medullary carcinoma of breast | 30 | 0 | 0 | 0 | 30 |
| Undifferentiated carcinoma of salivary gland | 7 | 0 | 0 | 0 | 7 |
| Large-cell carcinoma of lung | 48 | 0 | 0 | 0 | 48 |
| Anaplastic carcinoma of thyroid | 6 | 0 | 0 | 0 | 6 |
| Adrenal carcinoma | 6 | 0 | 0 | 0 | 6 |
| Parathyroid carcinoma | 2 | 0 | 0 | 0 | 2 |
| Apocrine carcinoma of breast | 3 | 0 | 0 | 0 | 3 |
| Sarcoimatoid urinary bladder | 5 | 0 | 0 | 0 | 5 |
| Carcinosarcoma of the uterus | 5 | 0 | 0 | 0 | 5 |
| Desmoplastic small round cell tumour | 3 | 0 | 0 | 0 | 3 |
| Benign entities | | | | | |
| Total | 398 | 0 | 0 | 0 | 398 |
| Thyroid adenoma | 47 | 0 | 0 | 0 | 47 |
| Parathyroid adenoma | 25 | 0 | 0 | 0 | 25 |
| Adrenal gland adenoma | 15 | 0 | 0 | 0 | 15 |
| Pleomorphic adenoma | 42 | 0 | 0 | 0 | 42 |
| Warthin's tumour | 26 | 0 | 0 | 0 | 26 |
| Oncocytoma of the kidney | 9 | 0 | 0 | 0 | 9 |
| Thymoma | 24 | 0 | 0 | 0 | 24 |
| Brenner tumour of ovary | 5 | 0 | 0 | 0 | 5 |
| Optic glioma | 1 | 0 | 0 | 0 | 1 |
| Craniopharyngeoma | 7 | 0 | 0 | 0 | 7 |
| Ganglioneuroma | 5 | 0 | 0 | 0 | 5 |
| Meningeoma | 49 | 0 | 0 | 0 | 49 |
| Schwannoma | 46 | 0 | 0 | 0 | 46 |
| Phylloides tumour of the breast | 10 | 0 | 0 | 0 | 10 |
| Mesothelial adenomatoid tumour | 7 | 0 | 0 | 0 | 7 |
| Benign apendegeal tumour, skin | 32 | 0 | 0 | 0 | 32 |
| Benign nevus, skin | 48 | 0 | 0 | 0 | 48 |
| Benign soft tissue tumours | | | | | |
| Total | 256 | 0 | 0 | 0 | 256 |
| GIST | 14 | 0 | 0 | 0 | 14 |
| Benign fibrous histiocytoma | 29 | 0 | 0 | 0 | 29 |
| Glomus tumour | 10 | 0 | 0 | 0 | 10 |
| Granular cell tumour | 3 | 0 | 0 | 0 | 3 |
| Tendon sheet, giant cell tumour | 34 | 0 | 0 | 0 | 34 |
| Haemagiopericytoma | 11 | 0 | 0 | 0 | 11 |
| Capillary haemangioma | 27 | 0 | 0 | 0 | 27 |
| Neurofibroma | 42 | 0 | 0 | 0 | 42 |
| Lieomyoma | 58 | 0 | 0 | 0 | 58 |

**Table 1** Continued.

| Organs | Negative | Weakly positive | Moderately positive | Strongly positive | Total |
|---|---|---|---|---|---|
| Lipoma | 27 | 0 | 0 | 0 | 27 |
| Angiomyolipoma | 1 | 0 | 0 | 0 | 1 |
| Premalignant entities | | | | | |
| Colon dysplasia | 137 | 0 | 0 | 0 | 137 |
| Mild | 43 | 0 | 0 | 0 | 43 |
| Moderate | 46 | 0 | 0 | 0 | 46 |
| Severe | 48 | 0 | 0 | 0 | 48 |
| Cervical CIN III | 28 | 0 | 0 | 0 | 28 |

AML, acute myeloid leukaemia; CIN III, carcinoma in situ; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumour; MALT, mucosa-associated lymphoid tumour; PNET, peripheral neuroectodermal tumour; RCC, renal cell carcinoma; TCL, T cell lymphoma.

(Dakocytomation, California, USA). 3,3-Diaminobenzidine tetrahydrocholoride was used as chromogen. In negative controls, a mouse serum was used instead of the primary antibody. Nuclear staining was required to consider Pax-5 staining as positive. At a double-head microscope evaluation of the IHC slides was performed semiquantitatively by two pathologists (PM-F and RS), who were not aware of the original histological diagnosis. The scores were reviewed, and whenever a discrepancy was noted between the first and second readings, a third pathologist (RP) reviewed the cases. The three pathologists reached an agreement on the final scoring. For scoring, intensity and percentage of positive cells were taken into consideration. The intensity was classified into three categories: weak, moderate and strong. The cut-off of ⩾5% positive tumour cells was used to define positive results.

## RESULTS

In normal human tissues, Pax-5 was only expressed in B lymphocytes of the lympho-haematopoetic organs, including lymph node, tonsil and spleen. All other tissues including the brain, bladder, cervix, endometrium, testis, ovary, pancreas, skeletal muscle, kidney, colon, stomach, skin and heart were negative for anti-Pax-5. Table 1 gives a summary of Pax-5 expression in 3758 benign and malignant tumour types.

Positive cases expressed Pax-5 in a nuclear pattern. On the other hand, Pax-5 was never expressed in a cytoplasmic pattern in any of the cases analysed. Pax-5 was expressed in 108/118 (91.5%) B-NHLs and in 60/70 (85.7%) HLs (fig 1A,B). In addition, all T cell lymphomas, consisting of four cases of anaplastic large-cell lymphoma and three cases of peripheral T cell lymphoma, were Pax-5 negative. In all, 24/34 (70.6%) MCCs, 38/48 (79.1%) SCCs of the lung and 3/5 (60%) SCCs of the urinary bladder expressed Pax-5 (fig 2A,B). On the other hand, Pax-5 was seen in 1/164 (0.6%) breast carcinomas, 2/204 (1%) endometrial adenocarcinomas and 1/452 (0.2%) urothelial carcinomas (UCs) of the bladder (fig 3A,C). All 398 benign tumours showed no expression of Pax-5 protein.

## DISCUSSION

By examining 3758 benign and malignant tumours, we found Pax-5 to be mostly expressed in B-NHL, HL, SCC and MCC. Furthermore, few cases of breast carcinoma, endometrial

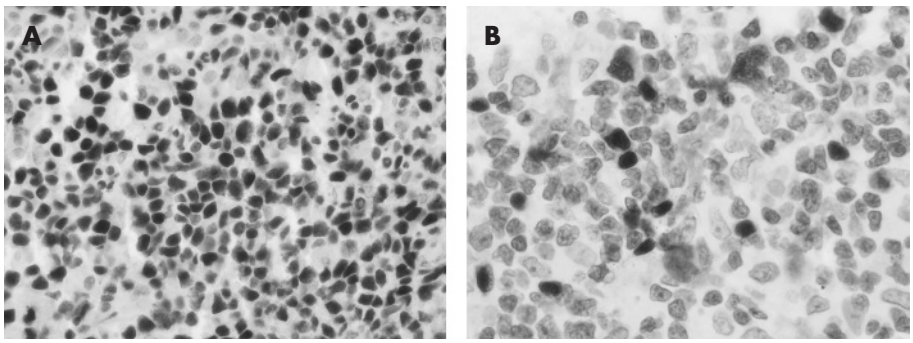


**Figure 1** (A) A case of diffuse large B cell lymphoma (×60). (B) Hodgkin's lymphoma, conventional type. Hodgkin's cell is shown in this image to stain for anti-Pax-5 (×40).

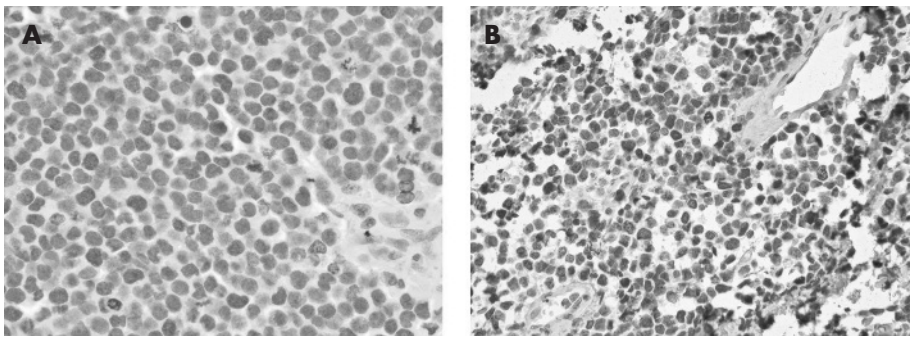


**Figure 2** (A) Merkel cell carcinoma (×60). (B) Small cell carcinoma of the lung (×40).

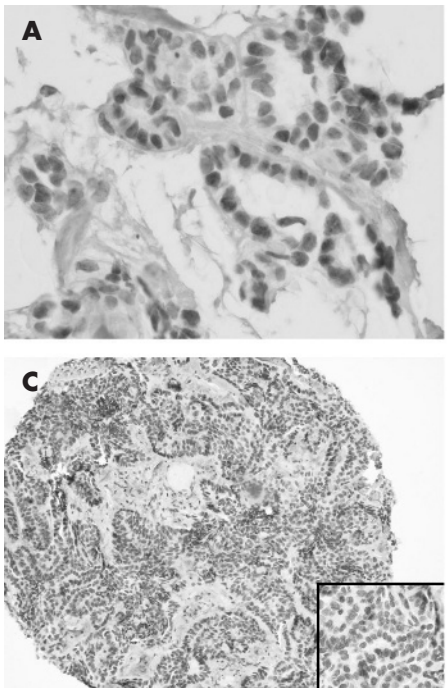


**Figure 3** (A) Ductal carcinoma of the breast (×40). (B) Endometrial carcinoma, endometrioid type. (C) Urothelial carcinoma of the bladder.

adenocarcinoma and UC of the bladder exhibited Pax-5. Pax-5 was not seen in any case of other type of malignancies, nor in any case of benign and premalignant tumours.

The expression of Pax-5 by lymphomas was well documented by Torlakovic *et al*.[17] After examining 592 cases of various lymphoma subtypes for Pax-5 immunoexpression, they found that 100% of B-NHL, 97% of HL and 0% of T cell lymphomas were positive. Accordingly, but with somewhat lower percentages compared with most of the published literature, we found Pax-5 expression in 91.5% B-NHL and in 85.7% HL. Pax-5 was not expressed in any case of T cell lymphoma. However, the common perception of Pax-5 expression being restricted to the B cell lineage changed when Dong *et al*[18] found Pax-5 expression in neuroendocrine carcinomas such as MCC (93.5%) and SCC (73.3%). The present study found that 70.6% of MCCs and 79.2% SCCs of the lung and the urinary bladder were Pax-5 positive. Yet, the main aim of this study was to explore the expression of Pax-5 in other tumour types. We found Pax-5 expression in few cases of UC, endometrial carcinomas and breast carcinomas. We did not find Pax-5 expression in any cases of the following small round cell tumours that can enter in the differential diagnosis of lymphomas and neuroendocrine carcinomas: germ cell tumours (0/82), rhabdomyosarcoma (0/13), alveolar sarcoma (0/1), endometrial stromal sarcoma (0/4), desmoplastic small round cell tumour (0/3), monophasic synovial sarcoma (0/3), liposarcoma (0/29), peripheral neuroectodermal tumours (0/16) and olfactory neuroblastoma (0/3). All the above findings strongly indicate that despite the expression of Pax-5 in few subsets of epithelial malignancies, Pax-5 is still considered as a good and reliable marker for B-NHL, HL, MCC and SCC.

An increased expression of Pax-5 mRNA using reverse transcriptase (RT)-PCR has been seen in astrocytomas, medulloblastomas and neuroblastomas. This overexpression seemed to be associated with tumour dedifferentiation.[11–13] In our study, glioblastoma multiforme (n = 50), astrocytomas (n = 48) and medulloblastomas (n = 5) were all negative for Pax-5. Furthermore, two recent studies investigating Pax-5 mRNA by RT-PCR in UC have emerged.[14 15] In those studies, Pax-5 was a frequent event in UC (79–83% of cases), leading the authors to believe that Pax-5 might have a role in the progression of UC. However, we were able to detect Pax-5 in just 1 out of 452 UC cases and with weak staining. Since no quantitative RT-PCR was performed in any of the above studies,[11–15] a plausible reason of this discrepancy could most probably be attributed to the low level of Pax-5 protein that is below the detection threshold of IHC. Another reason for this discrepancy could be the protein post-translational processing such as glycosylation or phosphorylation.

In summary, after analysing 3758 benign and malignant cases, we found, in accordance with previous reports, that Pax-5 is highly expressed in B-NHL, HL, SCC and MCC. In addition, Pax-5 was expressed in very few cases of breast, endometrium and urinary bladder carcinomas. Despite the presence of Pax-5 in subsets of epithelial malignancies, we concluded that Pax-5 remains a useful immunomarker for the diagnosis of B-NHL, HL and neuroendocrine tumours.

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### Take-home message

Despite its expression in few carcinomas, Pax-5 is still a useful marker to diagnose non-Hodgkin's lymphoma, Hodgkin's lymphoma and neuroendocrine tumours.

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