in 5 cases (25%), and medium-sized in 2 cases (10%). Results of immunohistochemistry were CD2 (60%), CD3ε (100%), CD4 (10%), CD7 (95%), CD8 (80%), CD56 (85%), TIA-1 (100%), Granzyme B (25%), TCRβ (10%), TCRγ (35%) and TCRγδ (50%). Double negative for TCR was found in 6 cases (30%). All cases were EBER-. The genomic profile showed recurrent copy number gains of 1q32.3, 4p15.1, 5q34, 7q34, 8p11.23, 9q22.31, 9q33.2, 9q34.13, and 12p13.31, and losses of 7p14.1. FISH showed 15 patients (75%) with a gain of 9q34.3 with good correlation with aCGH.

Conclusions: EATL in Japan is characterized by non-mono-morphic cells with a cytotoxic CD8+CD56+ phenotype similar to EATL type II. The genomic profile is comparable to EATL of Western countries, with more similarity to type I (gain of 1q and 5q) rather than type II (gain of 8q24, including MYC). The 9q34.3 gain was the most frequent change confirmed by FISH irrespective of the cell of origin of αβ-T cells and γδ-T cells.

128. BILATERAL ORBITAL PARANGLIOMA: A REPORT OF A UNIQUE CASE AND UPDATES ON ITS CLINICOPATHOLOGICAL FEATURES

Wan Faiziah Wan Abdul Rahman¹, Shatriah Ismail², Nassim Saremi³, Alfred K-Y. Lam^{3,4}

¹Department of Pathology, ²Department of Ophthalmology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia; ³Cancer Molecular Pathology and Griffith Health Institute, Griffith University, and ⁴Pathology Queensland, Gold Coast University Hospital, Gold Coast, Australia

Background: Orbital paraganglioma is a rare neuroendocrine neoplasm. The aim is to report a unique case of bilateral orbital paraganglioma and to systematically review the literature for clinicopathological features.

Methods: The unusual clinical presentation of a case of orbital paraganglioma was presented. In addition, a systematic review of the literature on orbital paragangliomas reported in the English literature was performed.

Results: A one-year-old Malay girl presented with bilateral leukocoria since 5 months of age with deterioration of vision. The clinical diagnosis of bilateral retinoblastoma was made. Chemotherapy was commenced before the left eye enucleation. Histopathological examination showed that the tumour was positive for neuroendocrine markers (synaptophysin, chromogranin and CD56) and negative for other markers (cytokeratins [AE1/3, CAM 5.2], vimentin, myogenin and GFAP). The morphology and immunohistochemical findings of the tumour were consistent with paraganglioma. A search of the literature revealed 29 cases of orbital paragangliomas and the present case was the youngest case being reported. The mean age at presentation of patients with orbital paraganglioma was 37. There was female preponderance (M:F=1:1.6) and slightly left laterality (R:L=1:1.2). Proptosis was the main presentation (79%). Surgical excision only through orbitotomy was the most preferred modality (62%) with 86% of them resolved without metastases or recurrence.

Conclusion: Paraganglioma of the orbit presented with bilateral leukocoria mimics retinoblastoma is very unusual. Detailed discussion on its clinicopathological characteristics may increase the understanding towards the correct diagnosis and the best therapeutic options.

129. HETEROTOPIC PANCREATITIS COMPLICATED WITH SEPTIC SHOCK: AN UNUSUAL PRESENTATION

Wan Faiziah Wan Abdul Rahman¹, Nur Asyilla Che Jalil¹, Hadif Samsudin², Siti Rahmah H.I. Merican³, Alfred K-Y. Lam^{4,5}

¹Department of pathology, ²Department of Radiology, ³Department of Surgery, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kota Bharu, Kelantan, Malaysia; ⁴Cancer Molecular Pathology and Griffith Health Institute, Griffith University, and ⁵Pathology Queensland, Gold Coast University Hospital, Gold Coast, Australia

Background: Heterotopic pancreas is defined as pancreatic tissue lacking anatomic or vascular communication with normal body of the pancreas. This entity is rarely symptomatic. The aim is to report an unusual presentation of inflamed heterotopic pancreas complicated with shock and multi-organ failure

Methods and result: A 62-year-old female presented with abdominal pain for one week duration associated with early satiety and repeated post-prandial vomiting. The biochemical tests were within normal range apart for the slight raised in serum amylase. CT scan of the abdomen and oesophagogastroduodenoscopy (OGDS) revealed a circumferential mass at the first part of the duodenum. Several days later, she presented with sign and symptom of shock. She was lethargic, impaired consciousness and had a poor oral intake. The blood pressure was 90/70 mmHg and the pulse rate was 120 beats/minute. The endoscopic biopsy result showed an inflamed heterotopic pancreas. Her condition deteriorated and subsequently died due to multi-organ failure.

Conclusion: Heterotopic pancreatitis presented with para-duodenal mass and complicated with shock is extremely rare. There is a potential of lethal complications when acute inflammation was noted in heterotopic pancreas.

130. MICRORNA-320d INHIBIT PROLIFERATION ABILITY OF DLBCL CELLS BY TARGETING CDK6

Jinfen Wang, Hong Su, Pengyan Wu
Shanxi Cancer Hospital, Taiyuan, Shanxi, PR China

Background: DLBCLs can be divided into two subgroups: germinal center B-cell (GCB) type and non-germinal centre-like (non-GCB) type; yet even within each subgroup, there are still wild variations in molecular characteristics and prognosis due to the heterogeneity.

Aims: To investigate the impact of miR-320d overexpression on DLBCL cell proliferation and analyze the possible molecular mechanism.

Methods: The target gene of miR-320d was predicted by TargetScan, miRanda and miRDB software analysis. The stably expressing miR-320d and CDK6-shRNA DLBCL cell lines (OCI-LY1) was created using lentiviral transduction. The CDK6 mRNA and protein levels were tested using qRT-PCR and western blotting, respectively. The proliferation ability of OCI-LY1/miR-320d and OCI-LY1/CDK6 shRNA cell lines was performed using CCK-8 experiments. In addition, Dual-luciferase reporter vectors were also constructed to detect the direct regulation of miR-320d target CDK6.

Results: miR-320d was predicted to target CDK6. miR-320d can inhibit the expression of CDK6 mRNA in OCI-LY1 ($p < 0.05$).

Compared to the control group, the CDK6 protein level was decreased in OCI-LY1/miR-320d group ($p < 0.05$). OCI-LY1/miR-320d group and OCI-LY1/CDK6 shRNA group cells' proliferation ability were markedly decreased compared to the control group ($p < 0.05$). Relative luciferase activity value of experimental group is lower than that of the control group ($p < 0.05$).

Conclusions: Up-regulation of miR-320d expression can inhibit the ability of malignant proliferation of DLBCL cell. The mechanism is concerned with that miR-320d could inhibit the expression of target gene CDK6 at the post-transcriptional level.

131. IMPROVED IMMUNOHISTOCHEMICAL METHOD FOR DETECTING ANTI-BCL-2 AND C-MYC ANTIBODIES IN MOUSE XENOGRRAFT MODELS

Xiaoling Xia, Jean Boyer, Liping Zhang, Burton Holmes, Rajalakshmy Ramalingam, Penny Towne
Ventana Medical Systems, Roche, USA

Background: Antigen detection is known to be affected by pre-analytical conditions such as fixative types, fixation time and delay to fixation. We used human breast carcinoma cell lines (BT-474 and ZR-75-1) generated xenograft tumors as model systems to analyze the impact of different pre-analytical conditions on BCL-2 and c-MYC staining. Antigen detection on mouse tissue is complicated by high level background staining due to the binding of secondary anti-mouse antibody to endogenous mouse tissue Igs and other components. We developed methods to use modified OptiView DAB detection system and unique linker methodology to almost completely eliminate non-specific staining and achieve satisfactory staining results.

Methods: Human breast carcinoma cell lines (BT-474 and ZR-75-1) xenograft tumors were fixed across a range of times in 6 different commonly used fixatives as well as delay to fixation with a range from immediate fixation to a 24 hour delay to fixation in 10% NBF. Two new IHC staining methods were developed to stain either anti-BCL-2 (124), a mouse monoclonal antibody or anti-cMYC (Y69), a rabbit monoclonal antibody, to compare with standard OptiView DAB detection system on a Ventana automatic staining instrument. An Abcam monoclonal rabbit anti-mouse IgG was employed as linker followed by a modified OptiView DAB system to detect BCL-2 expression. We also used a modified OptiView DAB detection system to measure c-MYC expression.

Results: Antigen detection with indirect immunohistochemical methods is hampered by high background staining if the primary antibody is from the same species as the examined tissue. This high background was eliminated by using an Abcam monoclonal rabbit anti-mouse IgG as a linker followed by a modified OptiView DAB detection system. New methods ensured us to achieve a more precise understanding of protein expression level. The xenografts tissues fixed with zinc formalin and Z-5 showed equivalent staining to 10% NBF after fixation for at least 6 hours. However, AFA, 95% EtOH, Prefer fixative did not perform equivalently over the range of fixation times when compared to 10% NBF, regardless of fixation time.

This study also demonstrated degradation of BCL-2 and c-MYC antigenicity in response to delay in fixation if tissue was left unfixed at room temperature for more than two hours.

Conclusion: Our study recommended that the best fixatives were 10% NBF, Zinc fixative and Z-5 between 6 hours and 72 hours for both anti-BCL-2 and anti-c-MYC antibodies. In addition, the tissues should be fixed within 2 hours after tissue collection. New IHC methods yielded minimum reduction of BCL-2 and c-MYC specific signaling. Using this method, the tissue sections were remarkably free of the background staining that is typically seen in mouse tissues with mouse antibodies. Hence, this procedure provides an improved, high sensitive method for detecting protein expression level loss due to inappropriate pre-analytical sample treatment.

132. RARE HISTOLOGICAL PATTERNS OF ANGIOFIBROMA OF SOFT TISSUE

Yuichi Yamada, Hidetaka Yamamoto, Kenichi Kohashi, Yoshinao Oda

Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka, Japan

Background: Angiofibroma of soft tissue (AFST) is a rare benign soft tissue neoplasm, which is characterized by fibroblastic cytology, prominent vascular pattern and novel fusion genes NCOA2-AHRR/AHRR-NCOA2 or GTF2I-NCOA2. AFSTs present a wide spectrum of morphology, making a challenge to diagnosis, thus we came to the cogitation to report the special histological features confirmed by genetic analysis.

Aims: The study aims to reveal the histological variety of AFST confirmed by genetic data.

Methods: We reviewed all the 274 cases diagnosed as solitary fibrous tumor/hemangiopericytoma (232 cases), undetermined tumors of fibroblastic differentiation (36 cases) and recently diagnosed as AFST (6 cases), and picked up the 12 cases histologically compatible with AFST. All the 12 cases were genetically analyzed by RT-PCR method, and immunohistochemical stains were performed for available cases.

Results: Highlighted histological findings were as follows; amianthoid fibers, ossification, lymphoid follicles, lymphoid cuff, hemosiderin deposition, aggregate of foamy histiocytes, cystic change, necrosis, and hemorrhage. Immunohistochemically, the tumor cells were positive for EMA (4/10 cases), desmin (4/10 cases), CD163 (6/6 cases), CD68 (3/6 cases), estrogen receptor (7/7 cases), progesterone receptor (1/6 case), D2-40 (4/7 cases) and STAT6 (1/6 case, weak nuclear stain), but negative for CD34, alpha-smooth muscle actin, muscle-specific actin, S-100 protein, pan-cytokeratin, beta-catenin, MDM2 and CDK4. AHRR-NCOA2 fusion gene was detected in 8 cases.

Conclusions: We revealed the unreported histological variation and immunohistochemical findings of AFST and confirmed them by genetic methods. It was suggested that AFST should be considered in the diagnosis of fibrous or fibrohistiocytic tumors with unspecified features.