บทฟื้นฟูวิชาการ

The role and the application of immunohistochemistry in soft tissue lesions

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In 1994, the World Health Organization reclassified soft tissue lesions into 15 categories as follows ;1)fibrous, 2) fibrohistiocytic, 3) lipomatous, 4) smooth muscle, 5) skeletal muscle, 6) blood and lymph vessels, 7) perivascular, 8) synovial tumor, 9) mesothelial, 10) neural, 11) paraganglionic, 12)extraskeletal cartilaginous and osseous, 13) pleuripotential mesenchymal, 14) miscellaneous, and 15) unclassified. The correct diagnosis is based on the integration of clinical, radiographic, and pathological finiding. The more common problems of soft tissue lesions are due to the various groups of morphology such as small round cell, spindle cell, etc. Thus ancillary techniques have been applied to narrow possibilities and reach the diagnosis. Special stains, immunohistochemisty, electronmicroscopy, cytogenetic and molecular genetics have been employed. However, laboratory resources to support molecular level examinations is limited. Immunohistochemistry has been used ,and, is approved for some specific mesenchymal diseases such as Ewing's sarcoma etc. Also, there has been the development of many new antibodies for different methods to improve immunoreactivity. Therefore, immunohistochemistry seems to be the easiest tool to apply when combined with clinical findings and microscopic appearance. Unfortunately, cross reactivity, aberrant immunoreactivity and artifacts commonly occur in this method. Thus careful sampling, evaluation and a panel

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of multiple and appropriate antibodies is basicaly required for soft tissue lesions. This topic proposes 7 groups of commonly used markers to applied for lesions and consist of 1) general markers, 2) epithelial markers, 3) muscle markers, 4) vascular markers, 5) histiocytic markers, 6) neural markers, and 7) other new markers. This attempts to divide the many antibodies into variable groups because of the large scope of soft tissue lesions and the different properties of each antibody. Thus before the application of particular antibodies with others as panel markers for soft tissue lesions, the pathologist should fully understand all details such as reactivity, cross reactivity, pattern of staining, aberrant expression, and non-reactivity.

Key words: *Immunohistochemistry, Soft tissue lesions.*

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ในปี1994 องค์การอนามัยโลกได้ปรับปรุงการแยกกลุ่มของกลุ่มโรคเนื้อเยื่ออ่อน (soft tissue lesions) ได้เป็น 15 กลุ่มโดยแบ่งออกเป็น1) ไฟบรัส 2)ไฟโบรฮิสติโอซัยท์3)ไขมัน 4) กล้ามเนื้อเรียบ 5) กล้ามเนื้อลาย 6) หลอดเลือดและท่อน้ำเหลือง 7) กลุ่มรอบหลอดเลือด 8) ซินโนเวียล 9) เมโสทีเรียล 10)นิวรัล 11) พาราแกงกลีโอนิค 12) กลุ่มกระดูกอ่อนและกระดูกภายนอกกระดูก 13) เพอริโพเทนเชียล มีเซนคัย 14) อื่น ๆ 15) กลุ่มที่ไม่สามารถจัดเข้ากลุ่มใดได้ การวินิจฉัยโรคจะขึ้นกับการนำลักษณะทางคลีนิค, การตรวจทางเอ็กซเรย์, และลักษณะทางพยาธิวิทยามาใช้ร่วมกัน ปัญหาที่พบเกิดขึ้นบ่อยในกลุ่มโรคนี้คือลักษณะทางรูปร่างของเซลที่ตรวจพบทางกล้องจุลทรรศน์ซึ่งมีรูปร่างแตกต่างกันเช่น เซลรูปร่างกลม, เซลรูปกระสวยเป็นต้น ดังนั้นการนำเทคนิคต่าง ๆ มาใช้ร่วมในการจำกัดและทำให้การวินิจฉัยแคบมากขึ้นและนำไปสู่การวินิจฉัยสุดท้ายจึงถูกนำมาใช้ประกอบด้วย การย้อมพิเศษ, การใช้อิมมูโนฮิสโตเคมี, เซลพันธุกรรมและระดับโมเลกุล แต่อย่างไรก็ตามวิธีการที่ทันสมัยโดยเฉพาะระดับโมเลกุลซึ่งการรองรับทางห้องปฏิบัติการมีค่อนข้างจำกัด จึงพบว่าอิมมูโนฮิสโตเคมีจึงได้ถูกนำมาใช้อย่างกว้างขวางและได้การยอมรับเพื่อที่จะนำไปสู่การวินิจฉัยสุดท้ายของโรคในกลุ่มมีเซนคัย (mesenchyme) เช่น Ewing's sarcoma เป็นต้น ในขณะเดียวกันการพัฒนาแอนติบอดี้ชนิดใหม่ในวิธีการและกระบวนการต่างๆ เพื่อที่จะพัฒนาศักยภาพของการเกิดปฏิกริยาตอบสนองมีความก้าวหน้ามากและประสพความสำเร็จ ดังนั้นจึงดูเหมือนว่าอิมมูโนฮิสโตเคมีเป็นวิธีหรือเครื่องมือที่มีความง่ายและสะดวกสบายมากที่สุดในการนำไปใช้ แต่วิธีนี้มีข้อเสียคือ การเกิดปฏิกริยาข้ามกลุ่ม (Cross reactivity), การเกิดการตอบสนองคลาดเคลื่อน (aberrant expression), และสิ่งผิดพลาดต่าง ๆ (artifact) ดังนั้นการเลือกสุ่มตัวอย่างเพื่อนำไปตรวจ ตลอดจนถึงการแปรผล ร่วมกับการใช้ชุดของอิมมูโนที่เหมาะสมเป็นสิ่งจำเป็นและต้องการพื้นฐานเพื่อใช้ในการวินิจฉัยโรคในกลุ่มนี้ บทความนี้ได้นำเสนอการตรวจหาโดยใช้ marker เป็น 7 กลุ่ม ซึ่งเป็นตัวที่จำเป็นและใช้บ่อยในงานกลุ่มนี้แบ่งออกเป็น 1) ทั่วไป 2) อีพิทีเลียม 3) กล้ามเนื้อ 4) หลอดเลือด 5) ฮิสติโอซัยท์ 6) นิวรัล 7) ตัวใหม่อื่น ๆ ความพยายามที่แบ่งแยกเป็นกลุ่มของmarker เนื่องจากกลุ่มโรคนี้ค่อนข้างใหญ่และกว้าง ประกอบกับแอนติบอดี้แต่ละชนิดมีคุณสมบัติที่แตกต่างกัน ดังนั้นก่อนที่พยาธิแพทย์จะนำแอนติบอดี้ชนิดใดมาใช้ร่วมกันเป็นชุดเพื่อการวินิจฉัยโรคในกลุ่มเนื้อเยื่ออ่อน พยาธิแพทย์ควรจะทำความเข้าใจและรู้รายละเอียดของแอนติบอดี้แต่ละชนิด เช่น การเกิดปฏิกริยาการตอบสนอง, การเกิดปฏิกริยาตอบสนองข้ามกัน, รายละเอียดของการติดสีของแอนติเจน, การเกิดการตอบสนองคลาดเคลื่อน และการไม่เกิดปฏิกริยาการตอบสนองเป็นต้น

Conventional histopathology is still the most significant modality for diagnosis in the field of surgical soft tissue pathology. However, correct diagnosis is based on the integration of clinical, radiographic, and pathologic findings. Over the past decades the classification of soft tissue tumors were according to nuclear configuration. Later it was subclassified by pattern of differentiation and biological patterns. In 1994 the World Health Organization classification was amended to attempt to make it more useful and meaningful. Basically, soft tissue pathology is divided into reactive, benign and malignant and is named based on the predominant cell type that is depended on the line of differentiation of the tumor. Thus there are 15 categories as follows: (1) fibrous, (2) fibrohistiocytic, (3) lipomatous, (4) smooth muscle, (5) skeletal muscle, (6) blood and lymph vessels, (7) perivascular, (8) synovial tumor, (9) mesothelial, (10) neural, (11) paraganglionic, (12) extraskeletal cartilaginous and osseous, (13) pleuripotential mesenchymal, (14) miscellaneous, and (15) unclassified. [1]

Many new techniques for immunohistochemistry have been developed to improve sensitivity by enhanced immunoreactivity, such as pretreatment of formalin-fixed tissue with proteolytic enzyme, lead or zinc salt. There are claims that the most useful technique to retrieve antigen and increase antigenicity is enzymatic predigestion at optimal time and methods (microwave heating and boiling of tissue in citrate buffer).[2] Despite many attempts, there is still no specific marker that absolutely specific for detection in soft tissue pathology. The common problem for immunohistochemistry is the cross reactivity of some antibodies by sharing of common epitopes, aberrant immunoreactivity and many artifacts. From this point the interpretation of immunohistochemistry should be strict and cautious to avoid incorrect conclusions such as edge artifacts, diffusion of some antigens into adjacent tissue, especially myoglobin diffusion from necrotic muscle to histiocytes; and factor VIII- associated antigen endocytosis by non-endothelial cells. Thus all processing for the representative tissue submission and slide selection to order immunostaining should be more careful. The assembly of any histopathological findings, including nuclear and growth patterns admixed with changes in tumors such as calcification, osteoid, chondroid formation etc., is neccessary for the differential diagnosis assessment. Nontheless, the utilization of immunohistochemistry with standard light microscope examination is useful to narrow the differential diagnosis. Consequently, this method attepts to resolve the differential diagnosis but it should be borne in mind that the panel antibodies study is now the best procedure to achieve the diagnosis. However, we have to accept that some sarcoma may have the same immunphenotype from cytogenetic and molecular genetic studies. Thus we have to emphazie on panel antibodies studies that will be discussed later.

At this time, the role of cytogenetic and molecular genetic studies seems to be more specific to define many problematic soft tissue tumors and play an important role in assessment of tumors that have poorly delineated morphology, aberrant or absent immunoreactivity. The non-random, recurrent chromosomal abnormalities and other aberrations of some specific tumors such as alveolar rhabdomyosarcoma, Ewing's sarcoma/PNET etc. Many reports of cytogenetic studies have recently been published and

the list is increasing.[3] The potential is that cytogenetic studies will progress and be successful in reaching final accurate diagnosis that may contribute to the next modalities for treatment and also improved prognosis of patient.

This topic is presented in order to apply the basic and advanced antibodies necessary for diagnosis of soft tissue lesions. Each antibody plays different roles in normal tissue and various tumors. Some types of tumors are increasing, such as solitary fibrous tumor, hemangiopericytoma, neuroblastoma and Ewing's sarcoma, etc. Over decades, soft tissue lesions still troublesome for diagnosis have similar morphology, such as small round cells, spindle cells, etc. Furthermore, a problem for the pathologist is the numerous types of soft tissue tumors. Antibodies will be discussed in detail later. Seven groups of markers are :

1. General markers
2. Epithelial markers
3. Muscle markers
4. Vascular markers
5. Histiocytic markers
6. Neural markers
7. Other new markers

Each marker will be demonstrated in the different types of tumors, depending on its properties. However, details of reactivity, aberrant expression, patterns of reactivity, percent of reactivity, and non-reactivity may be parallel within some markers.

1. General markers

1.1 Vimentin

Vimentin is one of the intermediate filaments. The intermediate filaments are characterized by size between thin microfilaments and thick microtubule filaments of 10 nm in size. They are divided into 5 subgroups consisting of cytokeratin, vimentin, desmin, glial fibrillary acidic protein and neurofilament. As we know, their expression is not directly to only one kind of cell or tumor.

Vimentin (MW 57,000) is associated with mesenchymal cells and tumors. Formerly, it was believed that can separate between mesenchymal and epithelial tumor. Unfortunately, many reports show an expression of this in melanoma and adenocarcinoma of adrenal, breast, endometrium, lung, liver, and kidney.[4,5] Although the limitation of utility of this antibody but the coexpression of cytokeratin and vimentin has been found. It consisted of epithelioid sarcoma,synovial sarcoma, desmoplastic small round cell tumor, rhabdoid tumor, and mesothelioma.[6-13]

2. Epithelial markers

2.1 Cytokeratin

2.2 Epithelial membrane antigen (EMA)

2.1 Cytokeratin

Currently, cytokeratin has been subclassified into 20 subtypes which the molecular weight is ranging from 40 to 67 kd.[14-15] Normally, they are divided into low and high molecular weight and further to acidic and basic subfamilies. Generally, they present in the cells as pair and was formerly used to define only the epithelial in origin. It also appears less frequently in various non epithelial neoplasm including Ewing's sarcoma, leiomyosarcoma, malignant fibrous histiocytoma (MFH), malignant peripheral nerve sheath tumor (MPNST), alveolar rhabdomyosarcoma, desmoplastic small cell tumor, epithelioid form of hemangioendothelioma, and angiosarcoma.[16-19] This problem ever proved that is real expression of protein and result from gene de-repression during tumor

progression.[20-21] Cytokeratin antibodies selectivity can be used to address specific problem for surgical diagnostic pathology. Practically, we use monoclonal cytokeratin antibodies such as pan-cytokeratin (MNF116, AE1/AE3), low-molecular weight cytokeratin (35βH11, CAM5.2, $K_{s20.8}$ etc.) and high molecular weight (34βE12).

2.2 Epithelial Membrane antigen

This is another epithelial marker that is face the same problems with cytokeratin. It also various notable expression in plasma cells, T- cell lymphoma, fibrohistiocytoma, rhabdomyosarcoma, and leiomyosarcoma.[22-24] The reactivity for soft tissue tumor is typically found as well as cytokeratin but focally and less intensity including synovial carcoma, epithelioid sarcoma, desmoplastic small round cell tumor, rhabdoid tumor, mesothelioma. Additionally, It is positive in perineurioma (membranous staining) that is remarkable negative for S-100 protein.[25]

3. Muscle markers

3.1 Desmin

3.2 Muscle specific actin (MSA) or Pan-muscle actin

3.3 Smooth muscle actin (SMA) or Alpha - smooth muscle actin

3.4 Myoglobin

3.5 MyoD1

3.6 Other muscle antigens

3.1 Desmin

Desmin,55 kd, is a component of cytoskeletal of cardiac, skeletal , and smooth muscle cells. The localization of desmin within skeletal muscle and smooth muscle for this is different. For skeletal muscle cell, it located at region of Z-bands between myofibrils that function as a binding material for the contraction.[26] In smooth muscle cell is associated with cytoplasmic dense bodies and subplasmalemmal dense plaque. Normally, desmin immunoreactivity can identify in myofibroblast, reticulum cell of lymph node, endometrial stomal cell, fetal mesothelium, stromal cell of fetal kidney, and chorionic villi. Thus the evaluation of desmin reactivity should be concern. Moreover, it is focally express in various spindle cell lesions that is not traditionally considered smooth muscle. This would be according to myofibroblastic differentiation such as fibromatosis, malignant fibrous histiocytoma, and myofibroblastoma. Currently, it also shows evidence in alveolar soft part sarcoma and desmoplastic small round cell tumor. That probably point toward the straited muscle in origin instead of the uncertainty of exact nature. The presentation of globloid or punctate paranuclear mass in immunohistochemical study of desmoplastic small round cell tumor is also correspond with ultrastructural feature.[6,9,26] However, the aberrant expression also detects in malignant peripheral nerve sheath tumor, liposarcoma, angiomatoid fibrous histiocytoma.[29-30]

The alteration of desmin expression frequently depends on effect of fixative to preserve antigen such as formalin, B5, Bouin's, ethanol, and Zenker's solution. Desmin is less crucially adventage in smooth muscle tumor and additionally exhibit different in different primary lesions. In either event of leiomyoma and well differentiated leiomyosarcoma can easily detect but infrequently express in high grade lesion (about half) and less reactivity in some location such as gastrointestinal tract.[31-34] In fact, several report attest negativity in smooth muscle neoplasms.[35-37]

Contrary with rhabdomyosarcoma, desmin can detects about 80-100%, even in undifferentiated

type.[38] The very primitive muscle cells also negative express for desmin. However, in the context of round cell tumor in young patient, it stills very useful. The positivity of desmin demonstrates the evidence of rhabdomyoblastic differentiation in rhabdomyosarcoma or in neoplasm with rhabdomyoblastic component such as triton variant of malignant schwannoma. Nevertheless, the combination of MSA (Muscle specific actin) and desmin are highly sensitive and specific marker for rhabdomyosarcoma. Regarding to leiomyosarcoma, the sensitivity increase from 45% for desmin only, or form 50% for MSA only, to 64% for both markers.

3.2 Muscle specific actin (MSA) or Pan-muscle actin

Actin family of contractile protein, molecular weight 42 kd, diffusely presents in mammalian cells. It consisted of 3 subtypes base on electrophoretic motility.

1. Alpha actin : there are 3 isoforms (alpha-skeletal, alpha- cardiac, alpha-smooth muscle)
2. Gamma actin : There are 2 isoforms (gamma -smooth muscle and gamma- cytoplasmic)
3. Beta actin : There is only 1 form (beta-cytoplasmic)

The gamma-cytoplasmic and beta actin localized within all cells but alpha and gamma - smooth muscle units are more specific.

Muscle specific actin(HHF-35) reacts with alpha and gamma unit. It lacks specificity since it appears in cardiac, skeletal and smooth muscle cells.[39] Additionally, it also demonstrates in pericytes, myoepithelial cells and myofibroblast.[40] MSA also presents in myofibroblast-containg neoplastic tissues such as fibromatosis, benign fibrous histiocytoma, mammary myofibroblastoma. Despite the excellent immunoreactivity in myoepithelial and pericytes but the malignant counterpart such as pleomorphic adenoma and hemangiopericytoma reveals different result of reactivity. However, mostly are negative and probably due to the transformation to malignancy that associated with alteration of actin isotopes.

3.3 Smooth muscle actin (1A4) or Alpha-smooth muscle actin

The Smooth muscle actin uses for demonstration of smooth muscle cells and also myoepithelial cells and myofibroblast.[41] However, it did not label normal skeletal muscle and tumor thereof. Nevertheless, it also establish in malignant fibrous histiocytoma and kaposi's sarcoma.[42,43]

3.4 Myoglobin

Myoglobin, oxygen-binding protein, presents in skeletal and cardiac muscle. It is not express in smooth muscle cells as well as malignant counterpart.[44] The myoglobin when compare with other muscle markes reveals less sensitivity but more specificity to detect rhabdomyosarcoma. Generally, positivity of rhabdomyosarcoma for desmin and MSA is about 80% by using formalin-fixed tissue but only 45% expression for anti-myoglobin. The small amount of antigen expression perhaps insufficient for detection.[45] As we know ,desmin is synthesized early in the course of muscle development and persists thereafter. Co-expression of myoglobin soon follows and is associated with cross striation. Since from the study of fetal myogenesis showed the staining of desmin and myoglobin more uniform in fetal tissue than tumors, however, the staining of myoglobin was weak. Virtually, the level is increasing during development.

3.5 MyoD1

MyoD1, is a nuclear phosphoprotein (45kd),

which obtains from myogenic regulatory gene that include myogenin, myf-5, and mrf-4-herculin/myf-6.[46,47] This regulatory gene perform function since development and maintenance of embryogenesis. It seems to be present at all stages of skeletal muscle differentiation.The benefit is according to the appearance in both classical and pleomorphic rhabdomyosarcoma.[48] Eventhough, this one is the modern antibody, however, the paper to support of specificity is increasing.

3.6 Other muscle antibodies

It has shown previously that actin, desmin and myoglobin expression at earlier stage of muscle differentiation than troponin T and titin.[49,50] Thus titin and troponin T has found in more differentiated tumor. The rhabdomyosarcoma which had the different degree of differentiation such as alveolar, embryonal, and spindle cell rhabdomyosarcoma disclose the reactivity as follow; 25,50,and 100%, respectively.[51] The others antibodies that have been used include reagents that demonstrate sarcomeric myosin-fast and slow isoenzyme, Z-protein, isoenzyme of creatinine kinase.

4. Vascular markers

4.1 Factor VIII - associated antigen (Factor VIII - AG, von willebrand factor)

4.2 CD31 or Platelet-Endothelial Cell Adhesion Molecule; PECAM-1)

4.3 CD34

4.1 Factor VIII - associated antigen (Factor VIII - AG, von willebrand factor)

Factor VIII is synthesized by endothelial cell and also appears in various hematopoitic cells including platelets, mast cells, normal endothelial cell and endocardium. There is great variably expression of this antigen in angiosarcoma and when compare with benign vascular tumor and intermediate grade vascular tumor such as hemangioendothelioma is quite low. The percentage of demonstration is low or absence in higher grade angiosarcoma as well as kaposi's sarcoma.[52-54]

4.2 CD31 (platelet-endothelial cell adhesion molecule; PECAM-1)

CD-31 is a transmembrane glycoprotein and can demonstrate on the surface of endothelial cells as well as platelets, and plasma cells. This marker has higher benefit to detect angiosarcoma (80-100%) as well as benign vascular tumor.[55] However, some malignant mesothelioma, leiomyosarcoma, and carcinoma ever defined this antigen.[56] The conjunction with other vascular markers are essential for diagnosis vascular tumor.

4.3 CD34

CD34 antigen is a 110 kd glycosylated transmembrane protein.[57] It was recognized by two monoclonal antibodies, MY10 and QBEND10. Most studies show similar results and it's not necessary to pretreat tissue by microwave or enzymatic digestion except rare cases. Originally was used in field of hematopathology to defined myeloid leukemia. Currently demonstrates on non-hematopoitic tissue and also more specific for some mesenchymal tumors. Actually, this antigen can detects in hematopoitic stem cells, endothelial cells, endoneurial cells, and dendritic interstitial fibroblastic cells. It ever claimed in the past that also positive for normal adipocyte. However,this recently study of lipomatous lesions conclude that its reactivity obviously in spindle cell lipoma (18/18) and also some cases of dedifferentiated liposarcoma. The benign lipomatous lesions positive

only in spindle cell component not in the adipocyte.[58] Another recently description is myofibroblastoma of breast. So far,the list to define the restriction of this antigen seems to be expanding. Finally, the new list of potential CD34 positive lesions are leukemia(subset AML, ALL,Granulocytic sarcoma), Lymphoblastic lymphoma, Vascular neoplasm, Gastrointestinal stromal tumor (including epithelioid variant), Hemangiopericytoma, Epithelioid sarcoma, Solitary fibrous tumor, Dermatofibrosarcoma protuberans. Nevertheless, It's important to note that significant negative finding of CD34 in big groups of tumors which consisted of majority of carcinoma, melanoma, lymphoma (except lymphoblastic lymphoma), clear cell sarcoma, MFH, fibrosarcoma, synovial sarcoma, fibrous histiocytoma, rhabdomyosarcoma, alveolar soft part sarcoma etc. (Table 1)

It should be recognize that CD34 will not use as single antibody to detect this specific tumor. It rather considers to use as panel multiple markers studies such as epithelioid sarcoma that is only 57% reactivity for CD34.[59-60] Thus a negative reaction with CD34 can not exclude this entity if it positive for keratin, vimentin and epithelial membrane antigen. The significant number of positive cases for CD34 positive lesions are demonstration as following lists. (Table 2)

Focus on vascular lesions, CD34 predominantly express and defined as an effective marker to detect tumor with vascular differentiation that will more effectiveness when combine with CD31 and factorVIII-AG. Benign vascular tumor encounters high number of expression in most cases.[74] For the malignant counterpart,it also usefuls to defined angiosarcoma especially in high- grade lesion and show significant reactivity in epithelioid and solid variant of angiosarcoma.[60,62,75] The spindle cell area of kaposi's sarcoma evenly express this antigen (83/89 cases).[59,62,69,76,77] There is concordant expression with factorVIII-AG in epithelioid hemangioendothelioma but non reactive in spindle cell type.[59,61,62,68,78,79]

Table1. Summarization of new lists of CD34 reactivity in various soft tissue lesions.

CD34 positive lesions	CD34 negative lesions
Leukemia (AML, ALL, Granulocytic sarcoma)	Carcinoma
Lymphoblastic lymphoma	Melanoma
Vascular neoplasm	Lymphoma (except lymphoblastic type)
Gastrointestinal stromal tumor (including epitheliod variant)	Clear cell sarcoma
Hemangiopericytoma	Malignant fibrous histiocytoma
Epithelioid sarcoma	Fibrosarcoma
Solitary fibrous tumor	Synovial sarcoma
Dermatofibrosarcoma protuberans	Fibrous histiocytoma, Rhabdomyosarcoma
	Alveolar soft part sarcoma

Table 2. CD34 reactivity in soft tissue tumors.

Soft tissue tumors	Number of cases	Percent of Reactivity	References (No.)
Dermatofibrosarcoma, Protuberan	52/55	95	61 - 65
Fibrosarcomatous (High-Grade) Dermatofibrosarcoma Protuberans	15/33	45	66
Epithelioid sarcoma	13/23	57	59-60
Gastrointestinal stromal tumor :Benign and malignant			
- Spindle cell type	62/91	68	67
- Epithelioid type	11/18	61	67
Hemangiopericytoma	6/9	67	59,62,68,69
	27/27	100	70
Solitary fibrous tumor	15/19	79	71
	6/7	86	72
Spindle cell lipoma	18/18	100	58
Myofibroblastoma	2/3	67	73

5. Histiocytic markers

5.1 Alpha-1-antitrypsin, alpha-1-antichymotrypsin, and muramidase (lysozyme),

5.2 CD 68

5.1 Alpha-1-antitrypsin, alpha-1-antichymotrypsin and muramidase (lysozyme)

These antigens are presumed to identify tumor of histiocytic in origin. However, they are not specific since can occuring in various sarcoma especially the pleomorphic sarcoma, carcinoma and melanoma. Possibly, it occurs from endocytosis from plasma.[80] Since, the lack of specificity thus the role of this antibody seems to be very limitation.

5.2 CD68

CD68, a pan-macrophage antigen,is a 110 kd protein. However, there are many clones of macrophage such as HAM 56,PG-M1 etc.[81] This antigen can be localized within tissue macrophages, granulocytic precursors within bone marrow, and in neutrophils. The murine antibody KP1/CD68 was derived from immunization with a lysosomal fraction of pulmonary macrophages and may be constituent of lysosomes. The expression of KP1/CD68 macrophage associated antigen was study very extensive and found relatively wide spectrum within malignant neoplasm. (Table 3) The consensus of the presentation of this antibody may not true histiocytic origin and probably reflect lysosome from phagocytic activity. The specificity of this marker to diagnosis malignant fibrous histiocytoma is still debate. However, many reports to evaluation of specificity are increasing and based on interpretation. The strong decorate of antibodies in pleomorphic and spindle cell areas within background of each type of MFH significantly identify and support

Table 3. CD 68 express in various tumors.[82,83]

Tumors	Number of case	Percent of reactivity
Angiomatoid fibrous histiocytoma	9/19	47.3
Malignant fibrous histiocytoma (MFH)	19/24	79.2
Melanoma	51/73	69.9
Malignant schwannoma	8/22	36.4
Liposarcoma	3/9	33.3
Non-Hodgkin's lymphoma	107/434	24.7
Leiomyosarcoma	8/37	21.6

the diagnosis of MFH.[84] Additionally, it can occurs in multinucleated or osteoclast-like giant cells within malignant fibrous histiocytoma and plexiform fibrous histiocytoma. According to the obscure of histogenesis of MFH, thus the panel of antibodies to characterize and excluding other tumors combine with the histologic pattern are the best modality to diagnosis.

6. Neural markers

6.1 S-100 protein

6.2 Neuron specific enolase (NSE)

6.3 Leu 7/ CD57

6.1 S-100 protein

S-100 protein, molecular weight 21 kd, is an acidic protein generallly identified in the central and peripheral nervous system. Although, various expression in normal and malignant are widely demonstrate including glial cell, schwann cell, chondrocyte, adipocyte, myoepithelial cell, melanocyte, histiocyte which include langerhans' cell and reticulum cell. Besides the previous list, some soft tissue sarcoma that is still controversial about histogenesis also exprss this antigen such as granular cell tumor, chordoma, clear cell sarcoma, and synovial sarcoma.[85-88] The neoplasms along with normal tissue that we already known the various reactivity of this antigen. However, the pattern and intensity of expression is vast difference and interesting. SinceS-100 protein alway decorates diffuse and strongly in schwannoma. But neurofibroma shows focally positivity. Malignant peripheral nerve sheath tumor (MPNST) for conventional type usually express scattered staining pattern and detect about 50-71%.[89-94] Contrary with epitheliod type of MPNST which express diffuse and strongly positive about 80% of tumor.[95] S-100 protein is also very sensitive to detect melanoma that always present more than 95%.[96] Additionally, it also decorates various intensity in another mesenchymal tumors that include extra-skeletal myxoid chondrosarcoma, extraskeletal mesenchymal chondrosarcoma, and liposarcoma.[97-100]

Considering the staining for S-100 pattern, basically can detect in both the nucleus and cytoplasm. However, they alway accept the positive stiaining in nucleus more than cytoplasm.

6.2 Neuron specific enolase (NSE)

Enolase is an enzyme that consisted of three diverse subunits. The alpha subunit is found in glial cell of brain. Thus it was termed neuron specific enolase. Since it widely distributes throughout body

in different isoenzyme, the utility is limit especially the polyclonal type. It commonly uses for neuroblastic and neuroendocrine tumors more than soft tissue tumor since it less specificity.[101-102]

6.3 Leu 7/CD57

Leu 7/CD 57 can reacts with neurofibroma, benign and malignant nerve sheath tumor.[90-103] This positivity generally use adjunct with S-100. It doesn't more advantage than S-100 and can be identified in non neuronal tissue including leiomyosarcoma, synovial sarcoma, extraskeletal myxoid chondrosarcoma, extraskeletal mesenchymal chondrosarcoma, desmoplastic small round cell tumor.[87,88,97-99,104]

7. Othe new markers

7.1 MIC2 gene product (CD99, p30/32^{MIC2}; HBA-71,O13,12E7)

7.2 CD117

7.1 MIC2 gene product (CD99, p30/32^{MIC2}; HBA-71, O13,12E7)

The MIC2 gene is maping to the pseudoautosomal region of X and Y chromosomes. The MIC2 gene product, is membrane glycoprotein which was identified first by using monoclonal antibody 12E7.[105] Another monoclonal antibodies that recognized the different epitopes of same molecular weight, 30-32 kD ,p30/32^{MIC2} are p30/32,HBA-71,O13 as different commercial. The antibodies which react with p30/32^{MIC2} was grouped within the CD99 cluster. The function of glycoprotein which located on the cell surface may be involves in cell adhesion.[106-107]

The expression in most human cell lines is focal but can over-expressed in Ewing's sarcoma and peripheral neuroectodermal tumors (PNET). The sensitivity of the different clones of MIC2 gene product for Ewing's sarcoma and PNET was studied. The 12E7, HBA-71, and O13 being positive in 90%, 95-98%, 100%, respectively.[108] The specificity of these antibodies show highly specific in O13.

The comparitive study of O13 with 12E7 and HBA-71 has been reported negative for rhabdomyosarcoma in O13 and focally positive for 12E7 and HBA-71 in embryonal rhabdomyosrcoma and alveolar rhabdomyosarcoma, respectively. All antibodies have distinctly negative reaction in neuroblastoma that is very useful to distinguish from Ewing's sarcoma.[109] Although, it's strong expression for Ewing's sarcoma/PNET but it's not a specific marker.Since they also express in various tumors eventhough in small number of cases including lymphoblastic lymphoma, acute lymphoblastic leukemia, rhabdomyosarcoma, poorly differentiated synovial sarcoma, ependymoma, islet cell tumor, adult and juvenile granulosa cell tumors, Sertoli-Leydig cell tumors.[110-112] The list of positive staining is expanding. Some malignant is not conclusion such as mesenchymal chondrosarcoma.[113-116] Nevertheless, all antibodies can demonstrate the negative result in neuroblastoma, melanoma, esthesioneuroblastoma, non-lymphoblastic lymphoma.

This non-specific reactivity should not cause the diagnostic problem for small round cell tumor (eg. Neuroblastoma, lymphoma-leukemia, rhabdomyosarcoma, neuroendocrine carcinoma etc.), if the panel of antibodies containing lymphoid, muscle, and neural markers are applied in conjunction with this antibody.

7.2 CD117

CD117, c-kit, is a transmembrane protein receptor that encoded by c-kit proto-oncogene. Normally is of value expression in acute myeloid leukemia.

Recenly report the newly trend to detect gastrointestinal stromal tumor either spindle or epithelioid variant about 85%. And claimed that is more sensitive than CD34. However,the role to identify tumor as well as CD34 is not parallel.[117]

Conclusion

We have to accept that sometime only light microscopy is insufficient to type or reach the diagnosis. The special stain, immunohistochemistry, electronmicroscopy has been performed. Today, there is an increasing number of ancillary techniques to aid in the diagnosis and classification of soft tissue tumors such as Fluorescence In Situ Hybridization Analysis and cytogenetic. However, the laboratory resourses to support studies still limit. Thus, the special clinicopathologic correlation is basically required to integration. Immunohistochemical evaluation of soft tissue tumors is based on panel of several antibodies approach that should be apporopiate with the clinical findings and microscopic appearance. We can divide in different context such as small round cell, spindle cell, epithelioid, and pleomorphic cell and then assemble with the expression of majority component or elements. Regarding to the role of immunhistochemistry shoud be remind that do not try to use just only one antibody to specify the diagnosis.

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