CHAPTER 10

FROM MORPHOLOGICAL TO MOLECULAR DIAGNOSIS OF SOFT TISSUE TUMORS

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Abstract: Cytogenetic discoveries of balanced translocations in soft tissue tumors have opened the way to molecular genetic definition of these translocations as gene fusions from the late 1980s. Many sarcomas are known to have such fusions, and the demonstration of the fusion transcripts in tumor tissue is of great value in specific diagnosis of synovial sarcoma (SYT-SSX), Ewing sarcoma (EWS-Fli1), clear cell sarcoma (EWS-ATF1), myxoid liposarcoma (FUS-CHOP), and other sarcomas. These translocations are believed to be disease-specific and pathogenetic forces, despite occasional observations to the contrary. Demonstration of SYT-SSX and EWS-ATF1 fusion assists in the diagnosis of synovial and clear cell sarcomas in unusual locations, such as the gastrointestinal tract, where these tumors occur with low frequency. Demonstration of sarcoma translocations and their fusion by different assays is well established; use of in situ hybridization is limited by availability of specific probes. In two exceptional instances, the same translocation and gene fusion occurs in two unrelated diseases: ETV6-NTRK fusion in infantile fibrosarcoma and secretory carcinoma of the breast, and ALK-TPM3 fusion in inflammatory myofibroblastic tumor and large cell anaplastic lymphoma. Thus, the target cell of the genetic change is an important factor to define the resulting disease. Activating mutations in two related receptor tyrosine kinases (RTKs), KIT, and platelet-derived growth factor receptor alpha (PDGFRA) is central to the pathogenesis of gastrointestinal stromal tumors (GISTs), and countering the mutational activation by specific tyrosine kinase inhibitors, such as Imatinib mesylate, is now standard treatment for metastatic GISTs. KIT exon 11 mutations (in frame deletions, point mutations, and duplications) occur in GISTs of all locations, whereas a characteristic exon 9 insertion–duplication AY502-503 is nearly specific for intestinal vs gastric tumors. In contrast, PDGFRA mutations are nearly specific for gastric GISTs, especially those with epithelioid morphology. Mutation type influences therapy responsiveness, but fortunately very few GISTs carry primarily Imatinib-resistant mutations. Secondary drug resistance acquired during Imatinib treatment based on new, Imatinib-resistant mutations is a major problem limiting treatment success. Loss of NF2 tumor suppressor gene in a biallelic fashion is believed to be central in the pathogenesis of neurofibromatosis 2 (NF2) associated and sporadic schwannomas and meningiomas. The mechanism

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includes nonsense or missense mutation in NF2 gene, and loss of the other NF2 allele as a part of losses in chromosome 22q. Schwannoma types may differ in their pathogenesis: gastrointestinal schwannomas lack NF2 changes suggesting a different pathogenesis. Intraneural and sclerosing perineuriomas display similar NF2 gene alterations as seen in meningioma, indicating a similar pathogenesis and molecular homology. Specific viral sequences of human herpesvirus 8 (HHV8) are diagnostic markers for Kaposi sarcoma (KS), and are absent in angiosarcoma. Despite discovery on simian virus SV40 sequences in mesothelioma as a possible pathogenetic factor, recent studies suggest that the presence of these sequences may be artifactual and based on common presence of some SV40 sequences as PCR contaminants.

Key words: Sarcoma, translocation, fusion transcript, gastrointestinal stromal tumor, mutation, KIT, PDGFRA, schwannoma, perineurioma, NF2, human herpesvirus 8

1. INTRODUCTION

The purpose of this chapter is to review examples of application of molecular pathology as a tool in the diagnosis of soft tissue tumors and understanding their pathogenesis, and discuss problems related to the application of molecular pathologic analysis. Examples of the different molecular changes include tumor translocations, mutational activation of oncogenes, tumor suppressor gene alterations, and presence of viral sequences.

2. TUMOR-SPECIFIC FUSION TRANSLOCATIONS IN SOFT TISSUE SARCOMAS

2.1. General comments

Cytogenetic studies form the mid-1980s and on revealed several recurrent translocations in soft tissue sarcomas, such as t(X;18) in synovial sarcoma, t(11;22) and others in Ewing family tumors, t(12;22) in clear cell sarcoma of tendons and aponeuroses, t(9;22) in extraskeletal myxoid chondrosarcoma, t(11;22) in desmoplastic small round cell tumor (DSRCT), t(2;13) in alveolar rhabdomyosarcoma, t(17;22) in dermatofibrosarcoma protuberance, and t(X;17) in alveolar soft part sarcoma, among others (Borden et al. 2003; Lasota 2003). Thus, typical translocations occur in most sarcomas that are composed of morphologically homogenous tumor cells with limited overall atypia. It is a great tribute to morphological pathologists that many of the earlier described tumor entities have been confirmed as distinct genetically defined entities with a specific translocation — in fact in every case, description of the cytogenetic changes was preceded by morphological definition of the tumor.

In contrast to many of the morphologically homogeneous tumors, no tumor-specific translocations are known for sarcoma types generally characterized by high degree of atypia, such as leiomyosarcoma, malignant peripheral nerve sheath tumor, pleomorphic liposarcoma, and malignant fibrous histiocytoma.

2.2. Molecular diagnosis of sarcoma translocations

Sarcoma translocations often lead to fusions between functional domains of two different genes, resulting in formation of pathologic fusion transcripts and ultimately fusion proteins many of which act as aberrant transcription factors and are key pathogenetic factors. Many of these genes encode for nucleic acid-binding nuclear proteins such as transcription factor, or other regulatory proteins.

Molecular diagnosis of sarcoma translocations is possible by PCR-based methods that detect gene fusions or their fusion transcripts. Although sensitive and often feasible in formalin-fixed and paraffin-embedded tissue, these methods may have a problem of false positive results due to cross contamination of DNA or cDNA templates, especially if nested amplification is used, because the sizes and sequences of the PCR-products are generally identical. Fusion transcript assays require recovery of RNA, because the breakpoints are either unknown or too variable to be practically detectable at the genomic DNA level by PCR-based methods. However, Southern blot analysis is feasible for detection of these gene rearrangements in genomic DNA from fresh or frozen tissue.

The fact that many sarcoma translocations involve the Ewing sarcoma (*EWS*) gene in 22q12 as one of the translocation partners, offers a unique approach for diagnosis by detection of the breakage of the *EWS* gene in a fluorescent in situ hybridization (FISH) assay by a "break apart probe" (Lee et al. 2005). Although this assay does not detect the specific fusion transcript and would not specifically identify the type of fusion, it can be very useful to distinguish tumors with EWS break from their mimics that do not have such a break, e.g., in the differential diagnosis of Ewing sarcoma and poorly differentiated synovial sarcoma, because the latter has normal configuration of the *EWS* gene.

Although in general, sarcoma translocations are disease specific, there are two intriguing examples of the same translocation and gene fusion being present in two unrelated tumor types. One of these examples is the ALK-TPM3 fusion, which has been detected in inflammatory myofibroblastic tumor and large cell anaplastic lymphoma (Lawrence et al. 2000; Cools et al. 2002). Another example is ETV6-NTRK3 fusion translocation in infantile fibrosarcoma that also occurs in secretory carcinoma of the breast, an uncommon variant of ductal breast carcinoma that has a predilection to young women (Knezevich et al. 1998; Tognon et al. 2002). These examples illustrate that a tumor translocation is not always disease specific and that the same molecular lesion can produce a different disease in a different target cell.

2.3. Synovial sarcoma

Synovial sarcoma fusions involving SYT-SSX1, SYT-SSX2, or very rarely SYT-SSX4 genes, are specific for this tumor. Biphasic variants typically involve

SSX1, whereas monophasic variants can have SSX1 or SSX2 type fusions. Although initially fusion involving SSX2 were believed to be associated with a better prognosis, more recent large studies cast some doubt on that (Ladanyi 2001; Ladanyi 2002; Guillou 2004).

Molecular diagnosis is useful in confirming a poorly differentiated synovial sarcoma, which morphologically and immunohistochemically can simulate other sarcomas, especially Ewing family tumors, often also being CD99 positive. The lack of epithelial markers in some of these tumors adds to diagnostic difficulty and need to perform molecular diagnostic studies.

Also, diagnosis of synovial sarcoma in unusual locations, such as stomach, may need support of an independent molecular diagnosis. Indeed, there are gastric tumors that are histologically, immunohistochemically, and molecularly identical with the peripheral synovial sarcomas. Apparently only 1 case of primary synovial sarcoma of stomach has been reported so far (Billings et al. 2000). We have identified six cases among tumors originally believed to be "gastric leiomyosarcomas." Long survival in some patients mitigates against metastatic nature of the tumor.

Minute synovial sarcoma of hands and feet <1 cm is a diagnosis that can meet skepticism of clinicians, because these tumors are typically clinically believed to be benign processes; we recently analyzed 21 such biphasic and monophasic tumors and demonstrated SYT-SSX fusions to support the unexpected diagnosis.

Some reports utilizing PCR-based detection have suggested that other tumors, especially malignant peripheral nerve sheath tumors, and even neurofibromas can also have SYT-SSX2 fusions (O'Sullivan et al. 2001). These reports have been met with skepticism and not confirmed by others. The apparent detection of synovial sarcoma fusions may have been result of differences in tumor classification, or false positive assay resulting from PCR template contamination. Convincing arguments against the occurrence of SYT-SSX fusion translocations in MPNST include lack of cytogenetic evidence for t(X;18) translocation in any of these tumors, and lack of reproducibility of such findings in other large series (Ladanyi et al. 2001).

What is synovial sarcoma, a sarcoma with truly epithelial differentiation: is it more closely related to a carcinoma or mesothelioma? If we look the expression of markers, synovial sarcoma epithelia has features of both: it often has calretinin and keratin 5 expression, similar to mesothelioma, although not WT1 (Miettinen et al. 2001a, b). In our experience, markers more typical of carcinomas, such as BerEp4 and occasionally CEA are also expressed, along with a complex array of epithelial mucins, such as MUC1, MUC2, MUC5A, and MUC6. Therefore, by expression of markers of epithelia and mesothelia, synovial sarcoma has unique hybrid features not present in any normal cell type.

2.4. Desmoplastic small round cell tumor

One of the more recent sarcoma entities, DSRCT, was discovered based on its distinct immunohistochemical profile by Rosai and his coworkers (Gerald et al. 1991). This intra-abdominal small round cell tumor in children and young adult was previously variably believed to be carcinoid tumor, neuroblastoma, Ewing sarcoma, or rhabdomyosarcoma. The tumor was discovered as a small round cell tumor with nested growth pattern with a desmoplastic stroma, and the tumor being simultaneously keratin-desmin and NSE-positive, showing multidirectional differentiation. Presentation in children and young adults as an intra-abdominal tumor made DSRCT also a cohesive clinicopathological entity (Gerald et al. 1991). Soon the t(11;22) translocation was discovered in, but it was found to be different from the t(11;22) of Ewing sarcoma by the breakpoint in chromosome 11 being in p13 in the short arm (and not in the q24 in the long arm, as in Ewing sarcoma). Soon followed identification of the breakpoints involving Wilms tumor gene (*WT1*) in 11p13 and *EWS* gene in 22q (Ladanyi et al. 1994); it took less than 3 years to proceed form the initial description of the entity to the discovery of its specific gene fusion and molecular pathogenesis. In this tumor, *WT1* gene (tumor suppressor gene in Wilms tumor) is highly expressed to be a diagnostic marker not present in other small round cell tumors (Barnoud et al. 2000).

Subsequent discovery of identical tumors in the pleural cavity (Parkash et al. 1995), and the fact that DSRCT shares a number of features, coexpression of keratins and desmin and WT1-expression with mesothelial cells, especially the fetal ones (van Muijen 1987), raises the intriguing possibility that this tumor typically occurring in the serous cavities, could be related to mesothelial cells and their tumors.

2.5. Clear cell sarcoma

Clear cell sarcoma of tendons and aponeuroses, originally described by Enzinger (1965), is a rare sarcoma of peripheral soft tissues usually occurring in young subjects in distal extremities, often in association with tendons. This tumor has some biologic relatedness to melanoma, although it differs from melanoma in some respects. Although gene expression profiles of clear cell sarcoma and melanoma have many similarities (Segal et al. 2003), the occurrence of typical translocation is specific for clear cell sarcoma.

The t(12;22) translocation with EWS-ATF1 fusion characterizes clear cell sarcoma. Intestinal clear cell sarcoma is an unusual mesenchymal tumor that occurs in both stomach and intestines. Some of these tumors contain osteoclastic giant cells, and in these, the presence of clear cell sarcoma translocation t(12;22) has been cytogenetically confirmed (Zambrano et al. 2003). In our experience, these tumors occur in a frequency of 1 for every 300 GI stromal

tumors. They are S100-positive and KIT-negative tumors that in contrast with peripheral clear cell sarcoma often lack HMB45-positivity.

3. ACTIVATING RECEPTOR TYROSINE KINASE MUTATIONS: GASTROINTESTINAL STROMAL TUMOR AS AN EXAMPLE

3.1. Introduction

RTKs are key molecules in signal transductions pathways, where a growth factor signal initiates activation of the receptor and transmission of the activation to cytoplasm and nucleus by a number of downstream signaling proteins. There are approximately 60 RTKs of different types (Pawson et al. 2002). A receptor activated in many carcinomas is human epidermal growth factor receptor (HER). The best known activated receptors in soft tissue tumors are KIT and PDGFRA in GISTs.

3.2. Background on gastrointestinal stromal tumors

GISTs are the most common mesenchymal tumors of the GI tract; they typically express KIT, and comprise a great majority of tumors previously classified as gastrointestinal smooth muscle tumors. GISTs occur throughout the GI tract, most commonly in the stomach and small intestine. These tumors are currently the best known examples of soft tissue tumors with activating RTK mutations. This finding has a great clinical significance because of the availability of effective KIT inhibitor, Imatinib mesylate, as an effective treatment for metastatic and unresectable GISTs (Demetri et al. 2002). Unfortunately, this success has been tempered by common development of drug resistance, by acquired new mutations and rarely by KIT gene amplification over 1–2 years after institution of the therapy (Chen et al. 2005; Debiec-Rychter et al. 2005).

3.3. KIT and PDGFRA as oncogenes

KIT and PDGFRA are structurally and evolutionally closely related type III RTKs. Both are growth factor receptors that are the starting points for receptor-initiated cell signaling pathways. Under normal circumstances, these receptors are activated (= phosphorylated) by binding of their respective ligands (stem cell factor for KIT and PDGF alpha for PDGFA), and they then activate downstream target proteins by phosphorylation in a cascade-like manner. Activation of the signaling pathways leads to changes in cellular motility, prevention of apoptosis, and promotion of cell proliferation (Ronnstrand 2004). Activating mutations cause activation of KIT signaling independent of ligand binding, and similar mechanism is operational with many other oncogenes.

Activating mutations in KIT and PDGFRA are driving forces in GIST oncogenesis. This hypothesis has been confirmed at several levels approaching the

fulfillment of criteria analogous to Koch's postulate for infections. First, most GISTs have either KIT or PDGFRA mutations, and second, introduction of mutation specific for GISTs in lymphoblastoid cell lines causes them to proliferate (Hirota et al. 1998). Third, transgenic mice with introduced GIST-type KIT mutations develop a GIST-tumor syndrome (Sommer et al. 2003; Rubin et al. 2005). The presence of KIT mutations in already very small tumors also supports the initiating role of mutation (Corless et al. 2002), although other factors are probably also involved. There are characteristic genomic losses in GIST, commonly those of copies of chromosome 14 and 22. Their specific role in pathogenesis is not yet clear (El-Rifai et al. 1996).

3.4. KIT mutations is GIST

Approximately 60–65% of GISTs have KIT mutations, and 15% have PDGFRA mutations that are mutually exclusive. KIT mutations are clustered in a small number of hotspots that include exon 11, exon 9, exon 13, and exon 17 in the order of frequency. PDGFRA mutations occur in homologous exon positions, including exons 12, 14, and 18; the latter is most commonly mutated in this gene. Common to these mutations is that they preserve the reading frame and produce a mutant protein that renders constitutional activation to KIT or PDGFRA. Most KIT mutants are responsive to Imatinib mesylate inhibitor treatment, but some, especially the rare exon 17 mutations are primarily resistant (Corless et al. 2004).

A great majority (>90%) of KIT mutations occur in exon 11 (juxtamembrane domain). The juxtamembrane domain mutations apparently disturb this alpha helical domain of KIT leading to constitutional KIT activation (Longley et al. 2001).

KIT exon 11 mutations include in-frame deletions most commonly involving the region of codons 555–560. Missense point mutations in the same range and almost exclusively involve codons 557, 559, and 560. Codon 576 in 3′ part of exon 11 is less commonly involved. Both types of mutation occur in GISTs of all sites. In gastric GISTs, tumors with KIT exon 11 point mutations have a better prognosis than those with in-frame deletions (Miettinen et al. 2005a, b, c), but in our experience, small intestinal GISTs there seems to be no similar prognostic difference between tumor with KIT exon 11 deletions vs point mutations.

Internal tandem duplications (ITDs) that add genetic material typically occur in the 3′ portion of exon 11, most commonly in gastric GISTs and very rarely in small intestinal tumors. Tumors with these mutations have a generally favorable behavior, and in the stomach, they often represent mitotically inactive and collagen-rich tumors (Lasota et al. 2003b).

KIT exon 9 mutation involving a two-codon insertion/duplication AY502-503 in the extracellular domain is a relatively rare but clinicopathologically distinctive KIT mutation. It occurs almost exclusively in intestinal vs gastric GISTs, and its frequency among the small intestinal GISTs is approximately 10% (Lasota et al. 2000; Lasota et al. 2003c; Antonescu et al. 2003). Tumors with this

mutation are not morphologically separable from other small intestinal GISTs. Although tumors with this mutation generally have an unfavorable prognosis, their behavior is not statistically different from small intestinal tumors with other types of KIT mutations in our experience. Therefore, the adverse prognostic of the tumors with the exon 9 mutations is related to the more commonly adverse outcome of small intestinal (vs gastric) GISTs.

3.5. PDGFRA mutations in GIST

PDGFRA mutations occur almost exclusively in gastric and not in the intestinal GISTs. These mutations have a strong predilection to gastric GISTs with epithelioid morphology (tumors formerly often designated as leiomyoblastomas), and their overall frequency may be as high as 15% of all gastric GISTs (Heinrich et al. 2003; Corless et al. 2004; Lasota et al. 2004). Unfortunately, antibodies for consistent immunohistochemical demonstration of PDGFRA by immunohistochemistry are not currently available.

Most commonly, PDGFRA mutations occur in exon 18 of PDGFRA, and a great majority of these are point mutations D842V. Less commonly, they involve exon 12, where in-frame deletions similar to those in the homologous KIT exon 11 occur.

GISTs with PDGFRA mutations have a generally favorable prognosis, which partly relates to the fact that gastric GISTs are more favorable than the small intestinal ones. Exon 12 deletions are rare; the number of reported cases does not allow reliable comparison between them and other mutants (Lasota et al. 2004). It remains to be seen how the differential gene expression profiles between KIT and PDGFRA mutant GISTs is related to different behavior of these tumors (Antonescu et al. 2004, Subramanian et al. 2004).

There is a very small group of gastric GISTs (<1%) that have PDGFRA exon 14 mutations. These seem to be associated with better prognosis than expected based on size and mitotic rate parameters (Lasota et al. in press).

3.6. Unusual GIST subgroups that lack KIT and PDGFRA mutations

There are at least two clinicopathologically distinctive subgroups of GISTs that lack KIT and PDFGRA mutations: GISTs in children and neurofibromatosis1 patients. Thus, the pathogenesis of these GIST subgroups is currently unknown.

GISTs in children are very rare. In this age group, GISTs occur almost exclusively in the stomach, and are more common in girls. Prognosis is unpredictable, and there is often a long course of disease, with metastases developing over 10–20 years or more; even patients with liver metastases can have a long survival (Prakash et al. 2005; Miettinen et al. 2005a, b, c). A small number of childhood GISTs occurs in connection with Carney triad, a syndrome-combining GIST with pulmonary chondroma, paraganglioma, or both (Carney 1999).

Neurofibromatosis 1 is the most common autosomal dominant disorder, representing the most common tumor suppressor gene syndrome (Viskochil 2002).

Multiple neurofibromas are typical, and development of malignant peripheral nerve sheath tumors is one of the major complications.

Of some reason, NF1 patients commonly develop GISTs, with the risk of this occurrence estimated to be over100-fold (Andersson et al. 2005). In this patient population, the clinicopathological spectrum of GIST is distinctive: occurrence of multiple, often small GISTs typically in the small intestine. These patients more commonly have a good prognosis, but of some reason, those with duodenal GIST have more frequently progressive disease (Miettinen et al. in press). The NF1 patients show diffuse Cajal cell hyperplasia that most likely is a precursor stage for GISTs, somewhat similar to that seen in patients with familial GISTs or transgenic mice with constitutional KIT mutations.

4. NF2 TUMOR SUPPRESSOR GENE ALTERATIONS IN SCHWANN CELL AND RELATED TUMORS

4.1. General comments on tumor suppressor genes

A number of genes regulating cellular growth and proliferation have been found contributing to tumor pathogenesis upon their inactivation. Such inactivation typically is biallelic, and mechanism of inactivation includes most commonly allelic losses and mutations. Examples of tumor suppressor genes include retinoblastoma gene, neurofibromatosis 1, and *NF2* genes. The original concept was devised by Knudson (1971) on hereditary retinoblastoma as an epidemiologically based hypothesis before the actual retinoblastoma gene alterations were discovered.

4.2. Neurofibromatosis 2 gene

NF2 gene is located pericentromerically in the long arm of chromosome 22q12. It encodes for a cytoskeleton-associated protein merlin (also known as schwannomin) that regulates the growth of schwann cells and related cells (Xiao et al. 2003). Patients with the hereditary NF2 syndrome typically have an inactivating NF2 mutation, leading to loss of one fuctional allele. Tumors developing in NF2 syndrome: vestibular schwannomas, meningiomas, and pilocytic astrocytomas, usually have somatic allelic losses of the other copy of NF2 protein, leading to the loss of both NF2 alleles in the scheme of a recessive tumor suppressor gene requiring inactivation of both alleles for loss of function. Type of NF2 mutation with different degrees of NF2 alterations varying from total loss to alteration is a factor determining the severity of NF2 disease (Ruttledge et al. 1996).

4.3. NF2 alterations in schwannoma and meningioma

In addition to being a typical feature of the NF2 syndrome-associated schwannomas, peripheral schwannomas and meningiomas have similar alterations as somatic changes: e.g., allelelic losses of chromosome 22q, including the

NF2 locus, and nonsense or missense mutations of NF2, rendering both NF2 loci nonfunctional (Baser et al. 2003).

4.4. Gastrointestinal schwannoma

Gastrointestinal schwannomas are rare, distinctive nerve sheath tumors specific to the GI tract. Especially in earlier days, these tumors were routinely confused with gastrointestinal stromal and smooth muscle tumors. However, these tumors are negative for KIT and positive for S100 protein. They usually occur in the stomach or colon in older adults, and have characteristic histological features, including peripheral lymphoid cuff, microtrabecular pattern, and focal nuclear atypia (Daimaru et al. 1988; Miettinen et al. 2001a, b). These tumors arise from the autonomic nervous system schwann cells in the walls of stomach or intestines. GI schwannomas have neither losses of NF2 allele nor NF2 mutations, indicating that their pathogenesis (and possibly proper classification, at least in terms of molecular pathology) is different form classical schwannomas (Lasota et al. 2003d).

4.5. Perineurioma: an NF2 mutant tumor

Perineurial cell tumors (perineuriomas) are rare nerve sheath tumors that feature perineurial cell differentiation. Perineurial cells are EMA-positive spindle cells that surround peripheral nerves and represent peripheral continuation of pia arachnoid meningeal cells. Perineuriomas are typically composed of slender, tapered spindle cells, in some cases forming onion skin-like formations, and in other cases forming meningioma-like patterns or trabecular infiltrates. One of their rare variants, intraneural perineurioma, is known to have losses in chromosome 22q, but NF2 mutations have not been analyzed (Emory et al. 1995; Brock et al. 2005). Sclerosing perineurioma is a distinctive, perhaps the most common variant of perineurioma that typically occurs in hands and fingers of young adults as a small nodule (Fetsch et al. 1997). Previously these tumors were considered fibromas or sclerosing glomus tumors.

Sclerosing perineuriomas demonstrate allelic losses in 22q, and they also have missense mutations in *NF2* gene, indicating that they have molecular homology with meningiomas (Lasota et al. 2001). However, similar mutations have not been found in other variants of perineuriomas suggesting genetic and perhaps conceptual heterogeneity.

5. VIRAL SEQUENCES IN SOFT TISSUE TUMORS

5.1. Kaposi sarcoma, angiosarcoma, and HHV8

The best documented example of viral presence and pathogenesis in human sarcoma is HHV8 in KS. This gammaherpesvirus occurs in endemic, as well as immunosuppression-associated KS, and it is more common in populations with

higher frequency of this tumor, e.g., in Mediterranean region. HHV8 is believed to be a key pathogenetic factor for KS by interfering with host tumor suppressor mechanisms (Moore et al. 2004).

It has been somewhat contested whether HHV8 is truly specific for KS. Some PCR-based studies have revealed it in some angiosarcomas (McDonagh et al. 1996), whereas others did not find this virus in angiosarcoma or other non-Kaposi type vascular tumors (Lebbe et al. 1997; Lasota and Miettinen 1999). Cross-contamination of PCR templates is a significant problem in demonstration of viral sequences that are generally identical between positive specimens and can lead to overreporting of viral sequences in PCR-based studies.

Immunohistochemical demonstration of HHV8 viral proteins, especially the latent nuclear antigen-1, is a practical way to assess the presence of these viral sequences. Studies on vascular tumors have found consistent immunoreactivity in KS. However, no HHV8-immunoreactivity has been found in angiosarcoma, hemangioma, and other vascular tumors, indicating that HHV8 virus is generally not present in non-Kaposi type of vascular tumors and that its demonstration is useful for the differential diagnosis between KS and non-Kaposi type vascular tumors (Cheuk et al. 2004; Patel et al. 2004).

5.2. Simian virus SV40 in mesothelioma

Several investigators have reported on simian virus 40 (SV40) sequences in malignant mesothelioma, and its possible pathogenetic role has been suggested (Pass et al. 2004). On the other hand, a recent report detecting SV40 sequences as common SV40 laboratory contaminants, based on their presence in commonly used plasmids, has raised the question whether the significance of SV40 findings in mesothelioma has been overestimated (Lopez-Rios et al. 2004). The role of SV40 as an etiopathogenetic factor for mesothelioma has also been questioned based on lack of increased mesothelioma risk in patients who received an SV40-contaminated adenovirus vaccine (Rollison et al. 2004).

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