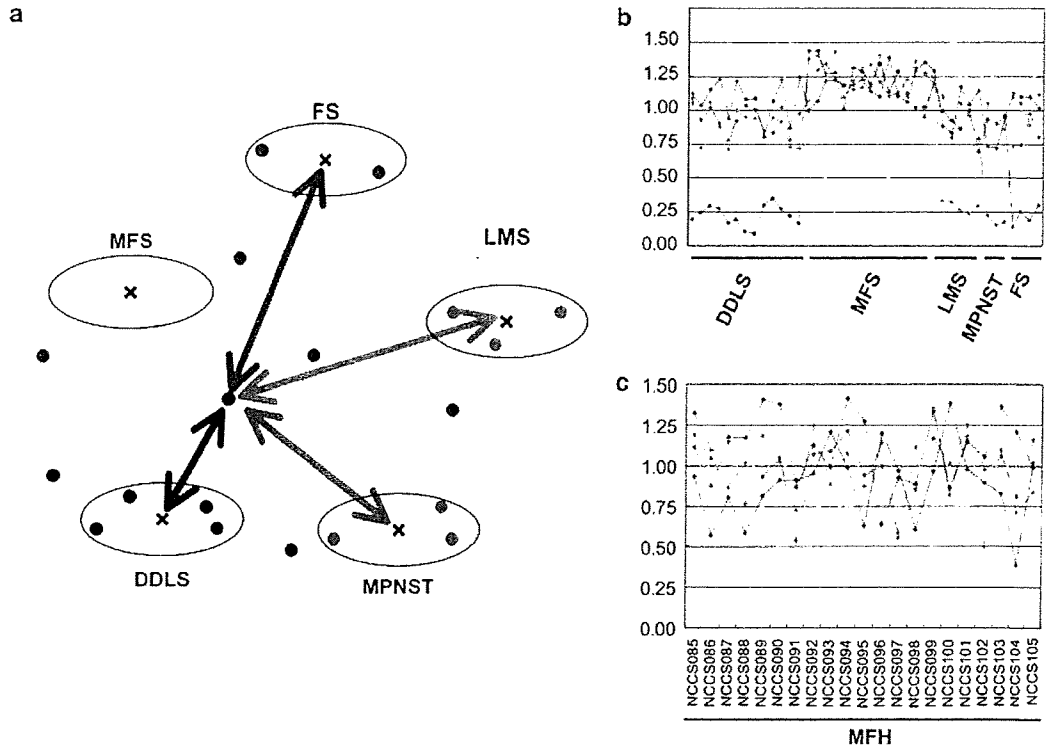

Figure 3 Distance evaluation of spindle cell and pleomorphic sarcoma samples from five sarcoma types. (**a**) Scheme of distance calculation. Each dot represents a sample colored according to its histology. Each × -mark represents the centroid of each histological type of sarcoma. Each arrow indicates the distance from a sample to a centroid colored by the histology. (**b**) Distances of 40 control samples from the five centroids. Note that the closest centroids matched their histology. (**c**) Distances of 21 MFH samples from the five centroids. DDLS, dedifferentiated liposarcoma; MFS, myxofibrosarcoma; LMS, leiomyosarcoma and FS, fibrosarcoma.

that did not fall into the appropriate cluster (Figure 2b). The top 50 probe sets with low *P*-values in each *t*-test were summed to obtain 248 probe sets (Supplementary data 6). On the basis of the expression of these 248 probe sets, the centroids of those five sarcoma types were calculated in advance, and inter-centroid distances and distances from five centroids to each control sample ($n=40$) were evaluated (Supplementary data 7 and Figure 3a and b). All inter-centroid distances were greater than 0.77 and the closest centroids for 40 control samples matched their histological types (Figure 3b), indicating that the evaluated distances were good indicators of sarcoma classification. We then evaluated the distances of each MFH sample from the five centroids (Figure 3c) and focused on determining the minimum (D_{min}) of the five distances. Small D_{min} values indicate high similarity to one of the five histological types in terms of gene expression. We used two cutoff values of 0.5 and 0.75 to evaluate similarity, because the majority of D_{min} values in control samples were less than 0.5 and most of the remaining four distances in each control sample were greater than 0.75. Among 21 samples, 3 showed marked similarity ($D_{min} \leq 0.5$), 12 showed moderate similarity ($0.5 < D_{min} \leq 0.75$) and the remaining 6 showed little similarity ($D_{min} > 0.75$). Among 15 MFH samples showing high or moderate similarity ($D_{min} \leq 0.75$), 6 were similar to myxofibrosarcoma, 5 to fibrosarcoma, 2 to MPNST and 1 each to dedifferentiated liposarcoma and leiomyosarcoma.

Histological Reviews

We re-examined the histology of 21 MFH samples with the knowledge of similarity to other types of spindle cell and pleomorphic sarcomas based on gene expression. Three MFH samples that showed high gene expression similarity ($D_{min} \leq 0.5$) displayed marked pleomorphism, indicating that a diagnosis of MFH was appropriate at the time of diagnosis. However, these samples also showed histological signatures of relevant subtypes. The NCCS099 sample, which was significantly close to the myxofibrosarcoma centroid ($D_{min} = 0.46$), showed prominent myxoid features very similar to myxofibrosarcoma in one third of the tumor (Figure 4a). The NCCS102 sample which was very close to the leiomyosarcoma centroid ($D_{min} = 0.50$) was positive for desmin and αSMA (Figure 4b–d). The NCCS104 sample, which was very close to the fibrosarcoma centroid ($D_{min} = 0.39$), showed focal

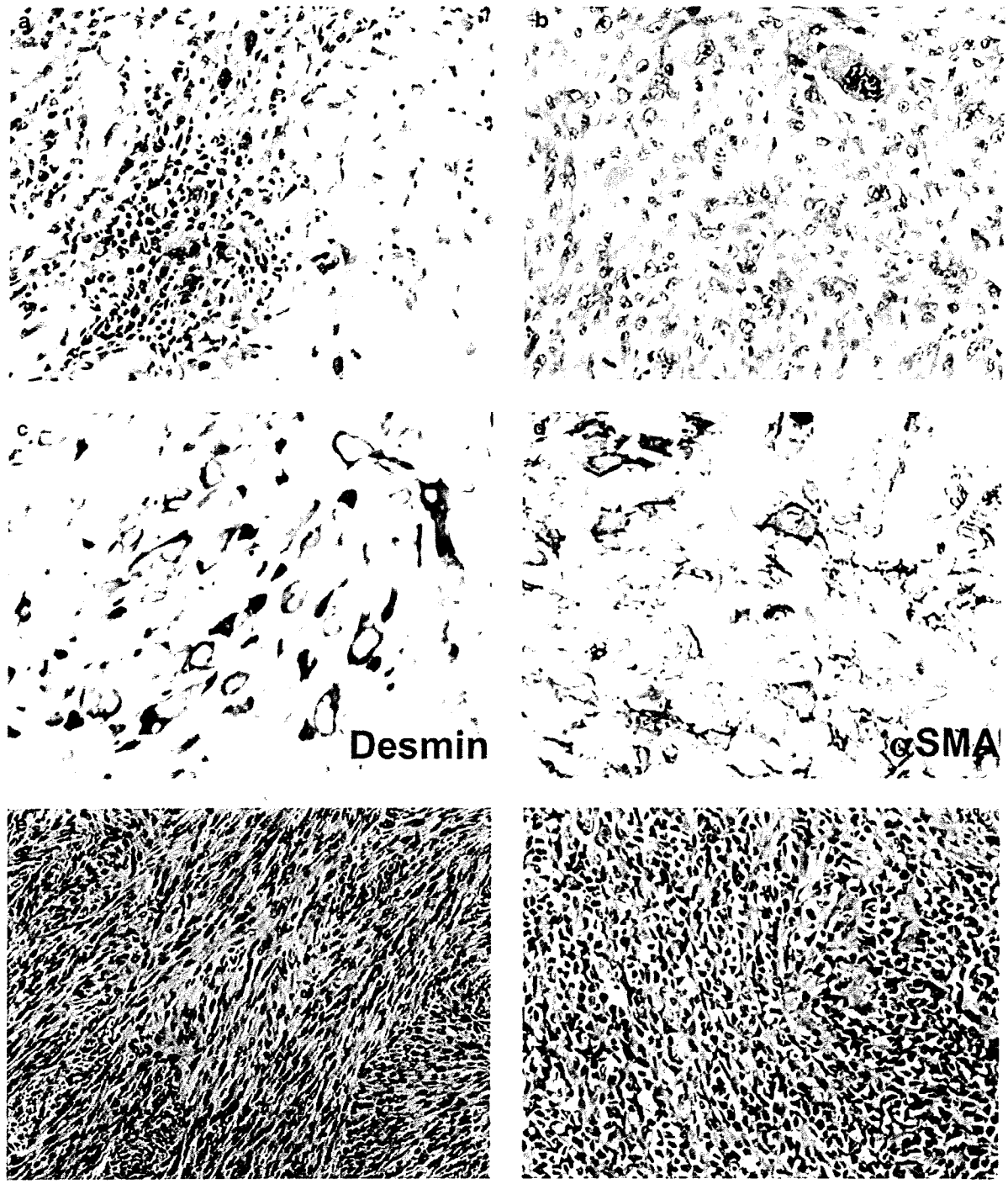

Figure 4 Histological review. (**a**) The border between pleomorphic area and myxoid area observed in the NCCS099 sample ($D_{min} = 0.46$ to myxofibrosarcoma) (hematoxylin and eosin stain). (**b–d**) Histology of the NCCS102 sample ($D_{min} = 0.50$ to leiomyosarcoma). This tumor showed marked pleomorphism (**b**) hematoxylin and eosin stain, but tumor cells were positive for desmin (**c**) and αSMA (**d**). (**e**) Fibrosarcomatous fascicular area seen in the NCCS104 sample ($D_{min} = 0.39$ to fibrosarcoma) (hematoxylin and eosin stain). (**f**) Epithelioid structure observed in the NCCS097 sample ($D_{min} = 0.56$ to MPNST) (hematoxylin and eosin stain).

fibrosarcoma-like herringbone and fascicular patterns by microscopic analysis (Figure 4e).

Among twelve samples showing moderate similarity to other types of sarcomas ($0.50 < D_{min} \leq 0.75$), the NCCS096 sample close to the dedifferentiated liposarcoma centroid ($D_{min} = 0.64$) was obtained from a recurrent sarcoma in the retroperitoneum. Although microscopic findings did not show

evidence of adipocytic differentiation or features of well-differentiated liposarcoma in regions adjacent to the tumor, the site of involvement suggested the possibility that the tumor originated from dedifferentiated liposarcoma. All five samples close to myxofibrosarcoma ($0.50 < D_{min} \leq 0.75$) showed scattered myxoid areas, but these findings were not sufficient to reclassify them as myxofibrosarcoma histologically. The NCCS097 sample, another pleomorphic sarcoma close to the MPNST centroid ($D_{min} = 0.56$), exhibited scattered whorled and epithelioid structures (Figure 4f) as well as tumor cells positive for cytokeratin, neurofilament and αSMA, indicating that this tumor had neuroectodermal differentiation. Its similarity to leiomyosarcoma ($D = 0.58$) would be reflected in αSMA positivity. For the NCCS091 sample close to MPNST and the other four close to fibrosarcoma, we did not observe any significant histological similarity to MPNST or fibrosarcoma, respectively. In summary, although more than half of the MFH samples ($n = 12$) were moderately similar in terms of gene expression to other sarcomas ($0.50 < D_{min} \leq 0.75$), only little resemblance was detectable by histological examination. Finally, the remaining six samples with high D_{min} values ($D_{min} > 0.75$) showed no identifiable histological similarity to the five sarcoma types (dedifferentiated liposarcoma, myxoid/round cell liposarcoma, leiomyosarcoma, MPNST and fibrosarcoma).

Genes Overexpressed in Myxofibrosarcoma

Diagnostically useful markers for myxofibrosarcoma are not well known. To search for candidate markers that genetically characterize myxofibrosarcoma, we selected upregulated genes by comparing myxofibrosarcoma samples ($n = 15$) with other spindle cell and pleomorphic sarcoma samples ($n = 25$). Three samples excluded from the previous analysis and 21 samples of MFH were not used for the marker search. From 11 300 probe sets, we selected 10 probe sets (five genes) with *P*-values < 0.001 based on the Student's *t*-test and more than five-fold greater expression (Table 1). Among them, expression of four probe sets (four genes) in respective spindle cell and pleomorphic sarcomas are shown in Figure 5a by the box-and-whisker plots. Since *ANK1* expression in MFH was much higher than that seen in myxofibrosarcoma (data not shown), its upregulation was not considered to be specific to myxofibrosarcoma. We performed quantitative RT-PCR with three other genes, *WISP2*, *GPR64* and *TNXB*, to verify the microarray findings (Figure 5b). Quantitative RT-PCR data confirmed consistent high expression of *GPR64* and *TNXB* in myxofibrosarcoma samples and in some MFH samples showing similarity to myxofibrosarcoma in terms of gene expression.

Discussion

An important aim of this study was to obtain new insights to classify a diverse group of soft tissue sarcomas. Our data showed that soft tissue sarcomas examined roughly fell into four groups (Figure 1a) (1) synovial sarcoma; (2) myxoid/round cell liposarcoma; (3) lipoma, well-differentiated liposarcoma with part of dedifferentiated liposarcoma and (4) spindle cell and pleomorphic sarcomas. Six histological types of spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST, fibrosarcoma and MFH) did not display distinct profiles but they shared a similar gene expression profile, forming a loose cluster in the hierarchical clustering analysis (Figure 1b). These results were broadly consistent with previous reports,[7,10] and histological similarity among spindle cell and pleomorphic sarcomas could be explained by similarities in gene expression. We could find some MPNST samples were located adjacent to the robust synovial sarcoma cluster in the hierarchical clustering analysis (Figure 1b), indicating that those MPNST samples shared similar expression patterns with synovial sarcoma as reported by Nagayama *et al*.[8] Our data also showed a common gene expression signature in synovial sarcoma and myxoid/round cell liposarco-

Table 1 Genes highly expressed in myxofibrosarcoma

Gene symbol	*Fold change*	P-*value*	*Description*	*Probe set ID*
WISP2	10.99	1.6×10^{-5}	WNT1 inducible signaling pathway protein 2	205792_at
GPR64	10.56	7.7×10^{-5}	G protein-coupled receptor 64	206002_at
TNXB	8.30	3.7×10^{-5}	Tenascin XB	208609_s_at
ANK1	7.03	1.8×10^{-5}	Ankyrin 1, erythrocytic	208352_x_at
S100A3	6.41	3.0×10^{-7}	S100 calcium binding protein A3	206027_at
ANK1	5.99	5.5×10^{-5}	Ankyrin 1, erythrocytic	205391_x_at
TNXB	5.96	2.9×10^{-5}	Tenascin XB	213451_x_at
TNXB	5.86	6.5×10^{-4}	Tenascin XB	216339_s_at
TNXB	5.74	1.3×10^{-4}	Tenascin XB	206093_x_at
TNXB	5.74	4.7×10^{-5}	Tenascin XB	216333_x_at

The top 10 probe sets with high fold changes were selected from 321 probe sets differentially expressed ($P < 0.001$ by Student's *t*-test) between myxofibrosarcoma samples ($n = 15$) and other spindle cell and pleomorphic sarcoma samples ($n = 25$) analyzed in Figure 3b.

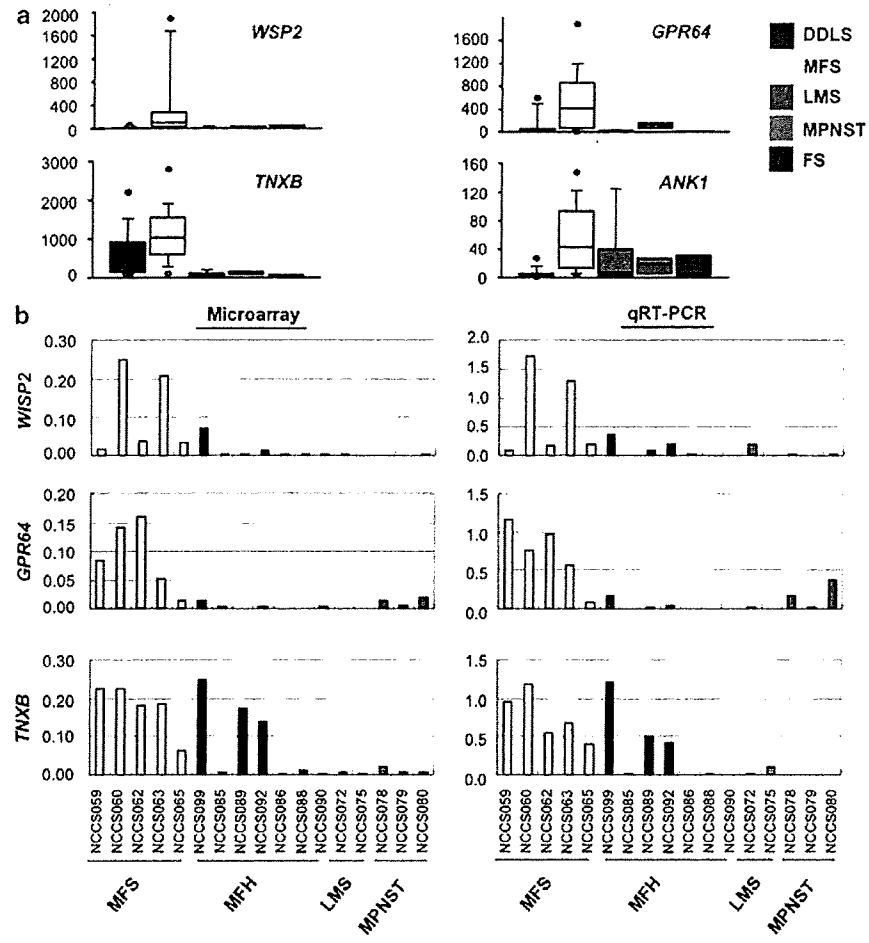

Figure 5 Genes highly expressed in myxofibrosarcoma. (**a**) Box-and-whisker plots indicating expression values for each histological type of spindle cell and pleomorphic sarcomas. A total of 40 control samples were analyzed. (**b**) Comparison between microarray analysis and quantitative RT-PCR of *WISP2*, *GPR64* and *TNXB* expression. Expression levels were normalized to that of *ACTB* in both microarray and RT-PCR data. NCCS099 ($D_{min} = 0.46$), NCCS085 ($D_{min} = 0.66$), NCCS089 ($D_{min} = 0.75$) and NCCS092 ($D_{min} = 0.66$) were similar to myxofibrosarcoma in terms of gene expression (see Figure 3c). DDLS, dedifferentiated liposarcoma; MFS, myxofibrosarcoma; LMS, leiomyosarcoma and FS, fibrosarcoma.

ma samples, distinguishing them from other tumors. Overexpression of genes encoding ribosomal proteins in myxoid/round cell liposarcoma was reported previously.[11] Another report showed that *SOX11*, *CTAG1*, *CTAG2* and *PRAME* were overexpressed in liposarcomas and absent or minimally expressed in all other tumors examined.[15] Among those genes, *CTAG1* and *PRAME* are both categorized as cancer testis antigens, and their expression in synovial sarcoma has also been reported.[16] Consistent with those reports, we found that *SOX11*, *CTAG1*, *CTAG2* and *PRAME* are highly expressed in both synovial sarcoma and myxoid/round cell liposarcoma. These similarities in gene expression may correlate with biological characteristics of synovial sarcoma and myxoid/round cell liposarcoma and suggest that these two sarcomas may share a common oncogenic pathway.

The so-called MFH was thought to be the most common soft tissue sarcoma in adults, and currently, it is widely accepted as a common morphological manifestation of a variety of poorly differentiated sarcomas. Re-evaluation of 'MFH' by different methods has been undertaken. Fletcher *et al*[5] reclassified 100 tumors primarily diagnosed as 'MFH' by histological methods and showed that the most common diagnosis was myxofibrosarcoma ($n = 29$), followed by leiomyosarcoma ($n = 20$). Hasegawa *et al*[14] examined immunoreactivity for smooth muscle markers from 100 samples of 'MFH' and reported that a large subset showed poorly differentiated smooth muscle or myofibroblastic features and should be regarded as pleomorphic leiomyosarcoma or pleomorphic myofibrosarcomas. Using comparative genomic hybridization, Derre *et al*[17] showed similar recurrent genomic imbalances in 'MFH' and leiomyosarcoma, and Coindre *et al*[18] reported that most inflammatory types of MFH developing in the retroperitoneum are identical to dedifferentiated liposarcoma. Here, we discussed

the possibility that 21 MFH samples could be reclassified into other types of spindle cell and pleomorphic sarcomas based on similarities in gene expression. For convenience of evaluation, we separated MFH samples into three groups according to the level of similarity to other sarcoma types. MFH with marked similarity ($D_{min} \leq 0.5$), MFH with moderate similarity ($0.50 < D_{min} \leq 0.75$) and MFH with no similarity ($D_{min} > 0.75$). Three samples very similar in gene expression to other sarcoma types ($D_{min} \leq 0.5$) resembled the corresponding histological types of spindle cell and pleomorphic sarcomas, and we concluded that these samples could probably be diagnosed as pleomorphic subtypes of those respective sarcomas based on current histological criteria. We then found that despite only marginal histological resemblance, more than half of the MFH samples (12/21) showed gene expression profiles similar to other sarcoma types ($0.50 < D_{min} \leq 0.75$). We considered that these moderate similarities in gene expression could correspond with pleomorphic change in each sarcoma type. Thus, although the samples cannot be diagnosed based on current histological criteria, it is possible to reclassify them as a pleomorphic subtype of those sarcomas based on gene expression. In this study, 40% (6/15) of reclassified MFH samples ($D_{min} \leq 0.75$) were similar to myxofibrosarcoma and 33% (5/15) were similar to fibrosarcoma, suggesting that a large subset of 'MFH' represents pleomorphic subtypes of fibroblastic sarcomas. Among the six cases of MFH similar to myxofibrosarcoma, five other than NCCS089 had deep-seated lesions, four (NCCS085, NCCS092, NCCS094 and NCCS101) had distant metastasis, and one (NCCS094) suffered local recurrence after surgery. Although the local recurrence rate (1/6) was unexpectedly low and distant metastasis rate (4/6) was high compared to canonical myxofibrosarcoma, these data could be consistent with the report showing that deep-seated lesions of myxofibrosarcoma were higher-grade, pleomorphic and large and increased the incidence of distant metastases.[19] About 30% of the MFH samples (6/21) did not show similarities to other sarcoma types ($D_{min} > 0.75$). One possibility is that '*de novo* undifferentiated pleomorphic sarcomas' truly exist. It is also possible that these samples represent advanced stage of dedifferentiation, which is beyond the analytical power of our study design. Another possibility is that the samples were derived from sarcomas of other differentiation not examined in this study. Extraskeletal osteosarcoma, rhabdomyosarcoma and other sarcomas could be the candidate. Reclassification accuracy should be improved by examining additional histological types of spindle cell and pleomorphic sarcomas.

Given that almost one third of MFH samples shared similar gene expression patterns ($D_{min} \leq 0.75$) with myxofibrosarcoma, we hypothesize that a large subset of 'MFH' may be pleomorphic subtype of myxofibrosarcoma. Myxofibrosarcoma is one of the most frequent sarcomas seen in late adults. However, little is known about its normal tissue counterparts, or factors underlying its extremely high local recurrence rate,[19] nor are there any good markers available for histological diagnosis. Identification of genes highly expressed in myxofibrosarcoma would offer an important clue to address these problems. Here, we found *WISP2*, *GPR64* and *TNXB* were upregulated in myxofibrosarcoma compared with other spindle cell and pleomorphic sarcomas. WISP2 is a member of the WNT1 inducible signaling pathway (WISP) protein subfamily, which belongs to the connective tissue growth factor family. WISP family members are secreted, cell- and matrix-associated proteins that play critical roles in cell differentiation and survival, wound repair, vascular disease, fibrosis and progression of certain cancers.[20–22] GPR64 is a highly conserved, tissue-specific heptahelical receptor of the human epididymis,[23–25] and there are no reports on the relationship of GPR64 to any type of cancer. TNXB is the largest member of the tenascin family of extracellular matrix proteins, which have anti-adhesive effects as opposed to the adhesion activity of fibronectin. It is expressed in musculoskeletal, cardiac and dermis tissue, and its deficiency is associated with the connective tissue disorder Ehlers-Danlos syndrome.[26–28] Although it is not clear if these genes play a role in myxofibrosarcoma, they may serve as novel diagnostic markers.

In this study, we primarily analyzed gene expression of MFH and other types of spindle cell and pleomorphic sarcomas (dedifferentiated liposarcoma, myxofibrosarcoma, leiomyosarcoma, MPNST and fibrosarcoma). Although these sarcomas showed a similar gene expression pattern and formed a relatively loose cluster, samples from five types of spindle cell and pleomorphic sarcomas were classified into respective histological types by excluding MFH samples. We identified genes that were differentially expressed among the five sarcoma types and could reclassify more than 70% of MFH samples into the five sarcoma types based on their similarities in gene expression using a combination of simple statistical analysis. These results suggest that gene expression profiling will be a useful tool to reclassify MFH and to aid histological diagnosis of a diverse group of soft tissue sarcomas. Although we cannot currently predict differences in clinical behavior of reclassified MFH due to the limited number of samples analyzed, accumulation of gene expression data should improve prediction of clinically important events, such as local recurrence, metastasis or therapeutic responses.

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Disclosure/conflict of interest

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Beyond foreign-body-induced carcinogenesis: Impact of reactive oxygen species derived from inflammatory cells in tumorigenic conversion and tumor progression

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Foreign-body-induced carcinogenesis is a traditional, maybe old, way of understanding cancer development. A number of novel approaches are available today to elucidate cancer development. However, there are things we learn from the old, and thus I bring out some examples of various clinical cases and experimental models of foreign-body-induced tumorigenesis. What is notable is that the foreign bodies themselves are unrelated to each other, whereas commonly underlying in them is to induce inflammatory reaction, especially stromal proliferation, where those exogenous materials are incorporated and undigested. Such foreign-body-induced carcinogenesis is also recognized in the step of tumor progression, the final step of carcinogenesis that tumor cells acquire malignant phenotypes including metastatic properties. And the phenomenon is universally observed in several cell lines of different origins. In this review I would like to show the evidence that tumor development and progression are accelerated inevitably by inflammation caused from foreign bodies, and that reactive oxygen species derived from inflammatory cells are one of the most important genotoxic mediators to accelerate the process.

Key words: inflammation; ROS; foreign body; carcinogenesis; tumor progression

The cancer research advanced in the last decade has pointed out that at least 5 cancer-causing factors exist: spontaneous replication errors in DNA, cytotoxic and/or inflammatory carcinogenic substances, genotoxic (direct DNA injurious) carcinogenic substances, irradiation including ultra violet and transduction of viral oncogenes. Infection/inflammation is unquestionably among them. In 1981, cancer epidemiologists estimated that around 10% of cancers was due to infection/inflammation in the Unites States.[1] And the most recent statistics show the major involvement of inflammation in the total cancer death, at various proportions among countries; for example, in 2005, the ratio of infection/inflammation to all the cancer death causes was around 25% in sub-Saharan Africa while that in Europe was around 6%.[2] In the year 2000, collectively, ~20% of all cancer deaths was attributable to inflammatory reactions due to chronic infections caused by infectious agents.[3]

In the same line of the evidence, foreign bodies incorporated into body for medical reasons or accidentally appear to lead to inflammation-based carcinogenesis. One of the epoch-making experimental studies in this aspect was established by Dr. Boone and his colleagues.[4–9] They observed tumorigenic conversion of immortalized cell lines after implantation of the cells attached to foreign bodies such as a piece of plastic plate or glass bead. Following their finding, we expanded the experiment to tumor progression, namely, the final stage of carcinogenesis acquiring malignant properties, and revealed that inflammatory-cell-derived reactive oxygen and nitrogen species were actually involved in the malignant progression. We also confirmed the universality of the phenomena in the cell lines of rat, mouse and human.

In the industrialized world, including developing and undeveloped countries alike, foreign-body-induced carcinogenesis tends to be poorly managed. A typical example is pleural mesothelioma among the particular workers in Japan; the disease is now belatedly being recognized after their years of exposure to asbestos in the past. Besides those, parasite-related or environmental carcinogenesis is among the foreign-body-induced carcinogenesis; however, in this article, I focus on the materials related with industrial products. I will present the past and present evidence for foreign-body-carcinogenesis and demonstrate that host inflammatory reaction is one of the major factors promoting carcinogenesis.

Foreign-body-induced carcinogenesis in human

Exogenously incorporated foreign bodies can induce tumors in human. Various materials are implanted in human body for prosthetic, reconstructive or cosmetic purposes today; or accidental implantation occurs, such as bullets or shrapnel at war; further, nondigestible particles and scarring may be added to this category of foreign body.[10–12] Tumor latency period from such implantation/injury till its detection is extremely long. It is estimated that over 25% of tumors appears in the span of 15 years and 50%, 25 years,[11] and the overall latent period in human is said to be 20 years.[10] It should be noted, however, that despite the increasing use of medical implants over the last 6 decades the frequency of tumor development there is extremely low. The critical difference of foreign-body-induced carcinogenesis from inflammation-based carcinogenesis is the low or rare tumor incidence, although the incidence depends on the composition of foreign body.

Particulate carcinogens

Particulate carcinogens are carbon black, asbestos, diesel exhaust, coal particle, acid aerosols, tobacco smoke and reactive gases such as ozone, sulfur dioxide and nitrogen oxides, and the most steady mechanism of particulate carcinogens is to elicit intense inflammatory responses. The conspicuous feature of inflammation in particle-induced carcinogenesis is the release of inflammatory cytokines and reactive oxygen species (ROS).[13] On the other hand, other mechanisms are also reported[13,14]; for example, such particles can act as carriers of carcinogen such as polycyclic aromatic hydrocarbons to increase retention period.[15]

Carbon black, a product of incomplete combustion of carbonaceous fuels and usually regarded as soot, was provisioned by Sir Percivall Pott in 1775 as a dreadful example of environmental carcinogen to cause scrotum tumors in chimney sweepers.[16] Soot itself appears to be carcinogenic on skin but possibly not on those of intracorporal organs such as respiratory tract epithelium.[17] A long-term cohort study revealed that carbon black or related by-products cause lung cancers in the workers at carbon black manufacturing factories.[18] Chronic exposure to particulate carcinogen increases tumor incidences to around 20%, with the majority of tumors appearing after 18 months of exposure.[19] While we are blessed with numerous products by technological innovations, regrettably some products are double-edged, contrary to the original aim of their production. The common features of the foreign bodies that induce carcinogenesis are listed in the later section. We need to pay attention to newly developed products before they

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appear in the market. Nano-scale particles, for example, are a great worriment because of the nature of their structures and those products are now spreading into every aspect of our daily life.[20]

Asbestos fibers

Asbestos is the commercial name for a group of hydrated magnesium silicate fibrous minerals, which is used as materials of cement, ceiling, automobile brake linings, and shipbuilding for their characteristic resistance to heat and combustion.[21] Asbestos is in 3 types of crystals: chrysotile (white asbestos), crocidolite (blue asbestos) and amosite (brown asbestos). The carcinogenicity of asbestos is believed to be related with the structural type rather than physiochemical properties.[22] Imports of asbestos increased in Japan from early 1960 and had a peak in 1974,[23] although there was general warning in 1973 that inhalation of asbestos causes pneumoconiosis, bronchial carcinoma, carcinoma of the gastrointestinal tract and mesotheliomas.[13,24] In parallel with asbestos consumption, the risk of developing mesothelioma (commonly pleural, and also peritoneal and pericardial mesotheliomas, depending on the distribution of mesothelium in body cavities) was belatedly recognized in people who had worked with asbestos. The mean latency of mesothelioma development is estimated at 20–40 years after the first exposure to asbestos.[21,25]

When asbestos is experimentally injected into a subcutaneous space in mice, extensive inflammatory reaction develops; surprisingly the subcutaneously injected asbestos fibers are transported into submesothelium of thorax and abdomen.[26] The conspicuous feature of asbestos-associated mesotheliomas is persistent inflammation[27] and active fibrosis[28] evoked by asbestos fibers since immune system cannot remove the nondigestible particles that lead to chronic inflammation (also termed "sterile inflammation").[29,30] It is considered that possible causes of asbestos-induced mutation are ROS, reactive nitrogen species (RNS) and their by-products. Asbestos fibers themselves catalyze the formation of hydroxyl radicals.[31,32] The production of hydroxyl radicals in cells after treated with crocidolite asbestos may result in the formation of premutagenic DNA basis, namely 8-hydroxy-2′-deoxyguanosine (8-OHdG).[33] Moreover, asbestos fibers stimulate infiltrated phagocytes to produce nitric oxide.[34] Thus in the environment of asbestos deposit site, RNS, in addition to generated ROS, may react with superoxide anion to produce peroxynitrites, which are able to oxidize guanosine to produce 8-OHdG. Unfried *et al.* have confirmed the evidence in *in vivo* situation that 8-OHdG-dependent mutagenesis in the mesothelium (*i.e.*, mostly G to T transversions) occurred in rats after injection of crocidolite asbestos.[35]

In Japan, an exponential increase of deaths from pleural mesothelioma was observed in the early 1990's. The mean annual number of deaths from malignant pleural mesothelioma was estimated at 500 between 1995 and 1999, and double-fold in 2005.[21] Considering the long latency period of mesothelioma, the incidence is expected to increase further in the coming decades.[36]

Metals

Kawanishi *et al.* have proposed the hypothesis that carcinogenicity of metal compounds is in 3 ways of inducing oxidative DNA damage.[37] First, metal generates ROS directly; second, ROS are generated through induction of inflammatory reaction by metal; third, metal activates carcinogenic chemicals. Inflammation-inducible metals are chromium (VI), cadmium, lead, cobalt (II), iron (III) and nickel compounds such as $NiSO_4$, NiO, Ni_3S_2 while Ni_3S_2 has a role to induce oxidative DNA directly in the presence of hydrogen peroxide.[37–39]

Medical devices

A wide variety of synthetic materials have been implanted in human since 1940's. In the middle to late 1960's, there were a few anecdotal reports, but they seldom mentioned the association between carcinogenicity and implantation of plastic materials. A long-term investigation of cosmetic breast surgery (breast augmentation) was the most typical example. Of 40,000 cases of implantation of polyvinyl alcohol sponges, breast cancer developed only in 6 cases (0.015%).[40] A cohort study revealed that the overall mortality rate was lower in women with breast implants than women with other plastic surgery or general population.[41] And in breast cancer statistics, no differences were found between women with breast implants and those without them in the disease stage at diagnosis, cancer recurrence or survival time.[42,43] Moreover, breast implant after mastectomy did not affect cancer-related mortality.[44] It is acceptable and certainly safe to implant foreign body for medical purposes by today's technology. Nevertheless, there are chances that implantation of foreign body is not always safe; *e.g.*, after arthroplasty of hip chromosomal aberrations are frequently found in the adjacent bone marrow.[45]

Scar cancer and mechanical trauma/wound-healing-associated cancer

Scars reactive to or encapsulation of foreign bodies is the fundamental and common host reactions (also known as fibrosis, desmoplasia or stromal proliferation). In early 1940's, G. Friedrich and R. Rössle found that lung carcinomas grew at the sites of fibrous scars in patients with smoking habit.[46,47] They observed that the scars had preceded the cancer, and thus termed it "scar cancer (Narbenkrebs)." Encapsulation is the oldest host adaptive immune system, and all the living things, from invertebrates to vertebrates, are equipped with the system.[48] In man, it is commonly associated with pathological situations and the typically described is fibrous tissue capsule or scar which surrounds foreign bodies. Histological examination shows that fibrous capsule is generally 300-μm thick around the implanted materials; however, no association is observed between the implanted materials and the degree of capsule reaction.[49] The initial pathognomonic sign is an emergence of extensively proliferating spots with a population of atypical cells, usually observed adjacent to a foreign body, and the cells are of polygonal and/or spindle cell types, expressing proliferating cell nuclear antigen.[49] These lesions can be considered as transitive to preneoplastic lesions.

There are 2 theories as for the induction of scar cancer. One is that the cells composing scar tissues convert themselves directly into tumorigenic ones; the other is that scar tissues secrete soluble factors that stimulate carcinogenic conversion or proliferation of tumor cells pre-existing around the scar. The former theory is supported by the evidence that tumor nodules arise in the serial transplantation of scar tissues which have originally been formed in the mice carrying T6 marker chromosomes; therefore by monitoring the chromosome as tag, one could detect the initial changes when the scar tissue exhibits conversion into malignant tumors at various degrees in the scar environment.[50,51] At this point, the latter theory is more acknowledged, because scar formation occurs along with tumor development, rather than before tumor formation, as seen in lung adenocarcinoma development, for instance.[52] Scar can promote malignant growth of tumor cells; for this reason, the minimum take dose of scarred tissue is much smaller than that of unscarred tissue, for lethal growth of transplanted tumor cells.[53]

Mechanical trauma (post-traumatic inflammation) or wound-healing processes show a similar trend to scar cancer. In 1920's a number of reports were published concerning the association between trauma/wound-healing and cancer, and some of them were the reports of case studies, showing that the incidence of trauma could cause cancer.[54,55] Trauma is considered not to act as initiator, but to act as promoter of carcinogenesis.[56] Fibrosis associated with tumor growth provides the environment to stimulate progression of tumor cells. Most of the clinical local recurrences of gastrointestinal tumors after resection of the primary tumor occur at the operated area of incision or anastomosis.[57,58] Whether fibrosis is accompanied with tumor or not depends on the types of collagen it produces.[59] Tumor-associated fibrosis is characterized by increased collagen Type III content; such fibrotic lesions are

mostly in the early immature stage. On the other hand, fibrosis unaccompanied with tumor contains decreased content of Type III and increased contents of Types I and IV collagen; such fibrotic lesions are mostly in the late mature stage.[60]

Foreign-body-induced experimental carcinogenesis

In 1941, Turner described the first experimental evidence for foreign body-induced sarcoma in rat using a disk of Bakelite[61] which was the first plastic made from synthetic components.[62] Brand *et al.* verified that carcinogenic potential depended on the properties of foreign body such as shape/size, smoothness, hardness, porosity and electrostatic load,[63] and was also influenced by gender and strain of the host.[64] Oppenheimer revealed that the tumor-forming material was not of degradable nature and had enduring smooth surface,[65] which was thereafter referred to as "Oppenheimer effect."[66,67] The effect is also called "solid-state tumorigenesis" because the carcinogenic conversion is brought about on solid surface of a foreign body.

The shape necessary for carcinogenesis as found in foreign bodies must be *in vivo* as well. An easily absorbable liquid would provide little or no chance for the conversion; however, if the liquid turns viscous, it can produce fibrosarcoma at the site of implantation.[67] The shape also influences tumor frequency; it is much higher in mice implanted with concave plastic discs than in those with convex ones because concave discs evoke more intense fibroblastic reaction.[10] Moreover, tumor incidence correlates with surface area of implants because the size of foreign bodies determines the degree of the inflammatory reaction.[10] Tumor formation seldom occurs when a small material has been implanted, but a large foreign body produces tumors constantly.[68] Textile materials, rough-surfaced implants and minced materials have little potential to induce tumors, whereas, if they are implanted, uninterrupted and smoothly-surfaced, they develop tumors.[10,28,69]

Porosity of the materials is influential. When implants are perforated and the holes are large, tumor incidence is reduced.[10,68] For instance, filters with pores of 0.22 μm or larger do not induce tumors whereas those of the same thickness with smaller pores will do. This is because the implants poorly develop connective tissue capsules and the pore is infiltrated with phagocytes in the former,[10] but the pore sizes below 0.22 μm are surrounded by thick collagen capsules and the filter pores are not infiltrated with phagocytes. The latter fibrous capsules are highly vascularized and the capillary loops develop close to the implant surface (less than 30 μm); on the other hand, angiogenic response is suppressed in the area of nontumorigenic filter implantation and the mean distances between the implant surface and capillaries remain 300 μm.[10] The filter with large porosity was found to have less cell proliferation, apoptosis, and fibrosis.[70] Moreover, hydrophobic filters develop more tumors than mice implanted with hydrophilic ones.[10] The electrical effects, interfacial forces or electrostatic and/or electrokinetic imbalance of implanted materials affect carcinogenic incidence.[53,69] Foreign materials positive for electric charge spontaneously attract inflammatory cells or easily form thrombus because most inflammatory cells, especially neutrophils, are negative for electric charge.[28]

It is of interest to know what cell types are responsible for foreign-body-induced carcinogenesis. There are 3 candidates, macrophages, fibroblasts and pericytes.[10] However, it would be appropriate to exclude macrophage as the early experiment suggested its irrelevance. Namely, in the experiment of bone marrow macrophage transplantation using distinguishable syngeneic mice, genetically marked sarcoma was not found in the mice whose bone marrow had been replaced to genetically tagged marrow cells.[10] Histologically the predominant cell type to form capsules around implants is fibroblast while foreign body-induced tumors are predominantly sarcomas. Fibroblast is a dubious entity. While development of fibrosarcoma is dominant in chemically-induced carcinogenesis, a variety of sarcomas appear in foreign-body-induced carcinogenesis: fibrosarcomas, malignant fibrous histiocytomas, pleomorphic sarcomas, myxosarcomas, hemangiosarcomas, rhabdomyosarcomas, osteosarcomas, leiomyosarcomas and mixed type.[49] This may illustrate that the initial mesenchymal stem cell reactive to foreign body is pluripotent. Pericyte is another candidate since its initial role is to support local angiogenesis. However, because of their pluripotent potential as a local mesenchymal cell population, they can convert themselves into sarcomas with various histological characteristics.[10]

Genetic alterations in the tumors arisen in the foreign body implantation have extensively been analyzed initially by karyotyping. Derivations from the normal chromosome number are seen in all the tumors studied[10]; morphological aberrations of chromosomes such as metacentrics or double minutes, and conspicuous abnormalities are recognized. Most karyotypes of foreign-body-induced tumors are exhibited in the hyperdiploid or the hypo- to hyper-tetraploid ranges.[10] Tazawa *et al.* identified some genes possibly responsible for the development of foreign-body-induced sarcomas.[71] They found that in 79% heterozygous p53-deficient ($Trp53^{+/-}$) mice with only 1 functional allele in the p53 gene developed spontaneous sarcomas after plastic plate implantation, whereas only 10% of mice with wild-type p53 developed sarcomas. They further demonstrated that the arising tumors have lost the remaining wild-type p53 allele, meaning complete loss of p53 function; this appears to be the underlying molecular mechanism during the development of sarcomas. That is, the tumor suppressor gene p53 is one of the genes responsible for foreign-body-induced carcinogenesis. p53 allele is inactivated by an increase of inflammation-mediated RNS.[71] Association between RNS and p53 mutations is evidenced in inflamed lesions of the colon with ulcerative colitis,[72] stomach,[73] brain[74] and breast.[75]

The susceptibility to foreign-body-related carcinogenesis does exist across species, and in strain- and gender-dependent manner.[10] Carcinogenicity of foreign bodies in human is rare, especially that of clinically used materials such as polyethylene, polyurethane, polyvinyl chloride, polymethylmethacrylate, silicone, titanium, nickel chromium, cobalt-chromium alloy and aluminum oxide; however, the same materials are indeed responsible for 25.8% incidence of sarcomas in 490 rats examined.[49] There is no association between histological types of sarcoma and implanted foreign materials, although polyurethane is the only material which tends to form hemangiosarcomas.[49]

Experimental rodent animals, except guinea pigs, exhibit high incidences of foreign body-induced carcinogenesis.[11] Yet differences do exist among the strains of mice in the incidence and the latency period.[64] And individuals genetically sensitive to foreign-body-induced tumors do exist in certain areas,[76] indicating differences in the sensitivity among races or groups of inhabitants. It is expected that there are labile gene(s) for this. Gender differences are also exhibited in mice. Latency period of tumor development is shorter in female mice than in male mice; however, the malignancy of the arising tumors is much enhanced in male mice.[10] By comparing the differences in sensitivity or resistance to foreign body-related carcinogenesis we might be able to find a clue to identify the molecules that control foreign-body-related carcinogenesis.

Experimental models of foreign-body-induced carcinogenesis and tumor progression

Serendipitous discovery of the foreign-body-induced carcinogenesis was made by Dr. Boone's group.[4–6] They showed that cells of nontumorigenic but immortalized mouse cell lines or freshly isolated connective tissues from mice were converted into lethally growing tumors of monoclonal origin in mice after they were implanted, being attached to foreign bodies.[4,5,8,9] They suggested at least 5 causes for the conversion: (*i*) immortalized cells acquired preneoplastic phenotype for the lack of anchorage-independent growth property, and thus the substrate-attached cells grew exponentially *in vitro* and lethally *in vivo*[4,5]; (*ii*) activation of endogenous oncornaviruses. However, this possibility was

low[6]; (*iii*) culture medium contained carcinogenic substances[7]; (*iv*) culture dish secreted carcinogenic substances.[8] Heppner, one of their contemporary researchers, speculated that inflammation was one of the possible causative factors for foreign-body-induced tumorigenesis.[77] Since then, we have expanded the experimental systems and demonstrated that foreign-body-induced inflammation and its-derived ROS are definitely the cause for the conversion.

To establish the experimental model of conversion, we chose the cells which were weakly- or nontumorigenic and nonmetastatic, but would grow *in vitro*, bearing the idea of xenogenization in mind. Xenogenization of tumor cells is the term meaning immunological spontaneous regression of tumor cells which had been infected with xenogeneic viruses,[78] transfected with the genes coding allogeneic antigen,[79] or exposed to mutagenic chemicals.[80] after injected into normal syngeneic host. Another approach to establish the model is to obtain the culture cell lines from a precancerous lesion.

Before I detail our unique animal models in which we can consistently observe not only tumor development but also malignant progression of regressive tumors or precancerous cells originated from rat, mouse and human, I should briefly trace the history of our experiments. Following Dr. Boone's work, Drs. Takeichi and Hamada expanded the concept of foreign-body-induced carcinogenesis to foreign-body-induced tumor progression. They obtained weakly tumorigenic and nonmetastatic clonal ER-1 cells by exposing SST-2 culture cell line, which had been established from a spontaneous mammary adenocarcinoma developed in SHR rat, to ethyl methanesulfonate *in vitro*.[81] The ER-1 cells regressed spontaneously after injected into syngeneic normal rats; however, if they were implanted, being attached to plastic plates (polystyrene, used as culture dish), into a subcutaneous space in rats, they acquired not only tumorigenicity but also metastatic ability.[81] The tumors arisen in rats showed various malignant phenotypes and their acquired phenotypes were stable. Thus they found that chronic inflammation was required for the progression of ER-1 cells. This model mimics inflammatory breast cancer in human, the most aggressive form of primary breast carcinoma with a dismal outcome despite multimodal treatment approaches.[82]

They also determined that fibrous stroma secreted soluble factors such as epidermal growth factor (EGF) and transforming growth factor-ß (TGF-ß).[83] The ER-1 cells were turned into invasive and metastatic tumor cells by EGF continuously added into the culture.[84] Those phenotypic changes depended on the duration of the EGF treatment; the malignant features were reversible during the 24-hr exposure to EGF, and after more than 4 weeks of exposure the acquired malignant phenotypes were completely fixed.[84] It has been confirmed that acquisition of malignant phenotypes can be prevented by addition of antioxidants, *N*-acetylcystein or selenium, into the medium,[84,85] and that 8-OHdG, a marker of ROS-mediated nucleic acid damage, is formed in the EGF-treated ER-1 cells; therefore, it is strongly suggested that the fundamental cause for the malignant progression is ROS generated by the fibrous stroma-derived growth factors.[84]

The mouse model has thoroughly been investigated, and reveals a definite connection between foreign-body-induced inflammation and carcinogenesis. For further experiments, we obtained regressive clonal QR cells by exposing clonal fibrosarcoma cells, BMT-11 cl-9, to a mutagen, quercetin *in vitro*.[80] Since QR cells grew progressively in immunosuppressed hosts, their regression was mediated by host immunity.[86] QR cells did not form tumors or metastasis after subcutaneous (2×10^5 cells) or intravenous (1×10^6 cells) injection into mice.[80] However, implantation of 1×10^5 QR cells attached to plastic plate[87] or injection into the preinserted gelatin sponge[88] in subcutaneous space of mice induced lethal tumors. Moreover, the arising tumor exhibited metastatic properties. The acquired various malignant phenotypes remained stable as far as examined for 1 year at least under cultivation *in vitro*.

We detected several gene alterations through the malignant conversion in this model.[89] The level of thymosin ß4 gene, which is known as an actin-regulating protein and to function for angiogenesis and wound healing,[90] was elevated in all of the arising tumor cells. From the results of sense and antisense cDNA transfection experiments, we determined that thymosin ß4 gene was responsible for tumor metastasis through regulating cell motility.[91] The expression of E1AF, a member of the *ets* oncogenic transcription factor, was found high in the arising tumor lines.[92] E1AF regulates tumor cell motility and invasive activities through induction of membrane-type 1-matrix metalloproteinase which converts the latent form of matrix metalloproteinase-2 into active form.[92] Thus E1AF makes tumor cells invasive.[92]

We used two foreign bodies in our decades of experiments. One was a piece of plastic plate and the other a piece of gelatin sponge which is used as hemostasis material during surgical operation. The difference between the foreign bodies is in the capacity to elicit inflammation. Plastic plate initially induces acute inflammation, which then changes to chronic inflammation, whereas gelatin sponge induces mild inflammation, and about 4 weeks after implantation it is naturally absorbed[93]; therefore such transition from acute to chronic inflammation is unlikely to occur in the use of sponge.[88] By using those foreign bodies, we modulated the quality and duration of the inflammation, and found that the type of inflammation needed for QR cells' growth and progression was acute-phase inflammatory reaction.[87,88,94] By histological examination, we found that neutrophils predominantly infiltrated the sponge.[93,94] In fact, one of the benefits of using gelatin sponge is that it is possible to collect the infiltrated inflammatory cells by treating the sponge with collagenase; the inflamed cells separated from the sponge can convert QR cells into tumorigenic ones if both cells are mixed and injected.[88,94] Namely, our primary observation was that foreign-body-reactive, early-phase inflammatory cells contribute to augment malignancy of QR cells.[87,88,94]

To test the role of neutrophils during inflammation, we eliminated neutrophils by administering anti-neutrophils antibody (RB6). Nearly all the arising tumors in the mice, nontreated or treated with control rat IgG, acquired malignant phenotypes. On the other hand, RB6 antibody-administered mice did not acquire malignant phenotypes.[94] We confirmed the results in integrin-β-2 knockout mice (C57BL/6J^{Itgb2tm1Bay} equivalent to CD18-deficient).[94] Integrin-β-2 is the key adhesion molecule for the migration of neutrophils into an inflammatory region. Neutrophil infiltration into gelatin sponge is abolished and acquisition of malignant phenotypes is suppressed in the integrin-β-2 knockout mice.[94] These findings show that neutrophils are one of the main components of inflammation-associated tumor development and progression. Interestingly, the capability of neutrophils to accelerate tumor cell malignancy depends on their activation phase since circulating or bone marrow neutrophils do not convert regressive tumor cells into malignant ones.[94]

It is reported that tumor-infiltrated neutrophils have the role to induce angiogenesis *via* MMP-9 secretion.[95] We assumed that the neutrophils also produced genotoxic substances since we detected somatic gene mutation in the coculture of QR cells and the infiltrated neutrophils.[96] The somatic mutation was inhibited in the presence of a radical scavenger, mannitol[96]; and in our previous study, immunostaining of 8-OHdG in the tumor tissues evidenced neutrophils' infiltration.[93,94] Therefore we examined the roles of neutrophil-derived ROS in this model. To determine the direct contribution of ROS to carcinogenesis, we used gp91phox gene knockout mice. Bactericidal function of neutrophils brought about generation of superoxide anions by forming NADPH oxidase complex ($gp22^{phox}$, $gp40^{phox}$, $gp47^{phox}$, $gp67^{phox}$, $gp91^{phox}$ and Rac1/Rac2) from interaction with cytochrome b_{558}.[97] The frequency of tumor development from the QR cells coimplanted with gelatin sponge was decreased in the $gp91^{phox-/-}$ mice. Moreover, acquisition of metastatic ability was reduced in the mice.[98] Thymosin ß4, a genetic marker of QR cell progression,[91] was not detected in the arising tumors in $gp91^{phox-/-}$ mice. To confirm whether phagocyte-derived ROS were actually involved in tumor progression, we isolated phagocytes from wild-type mice and

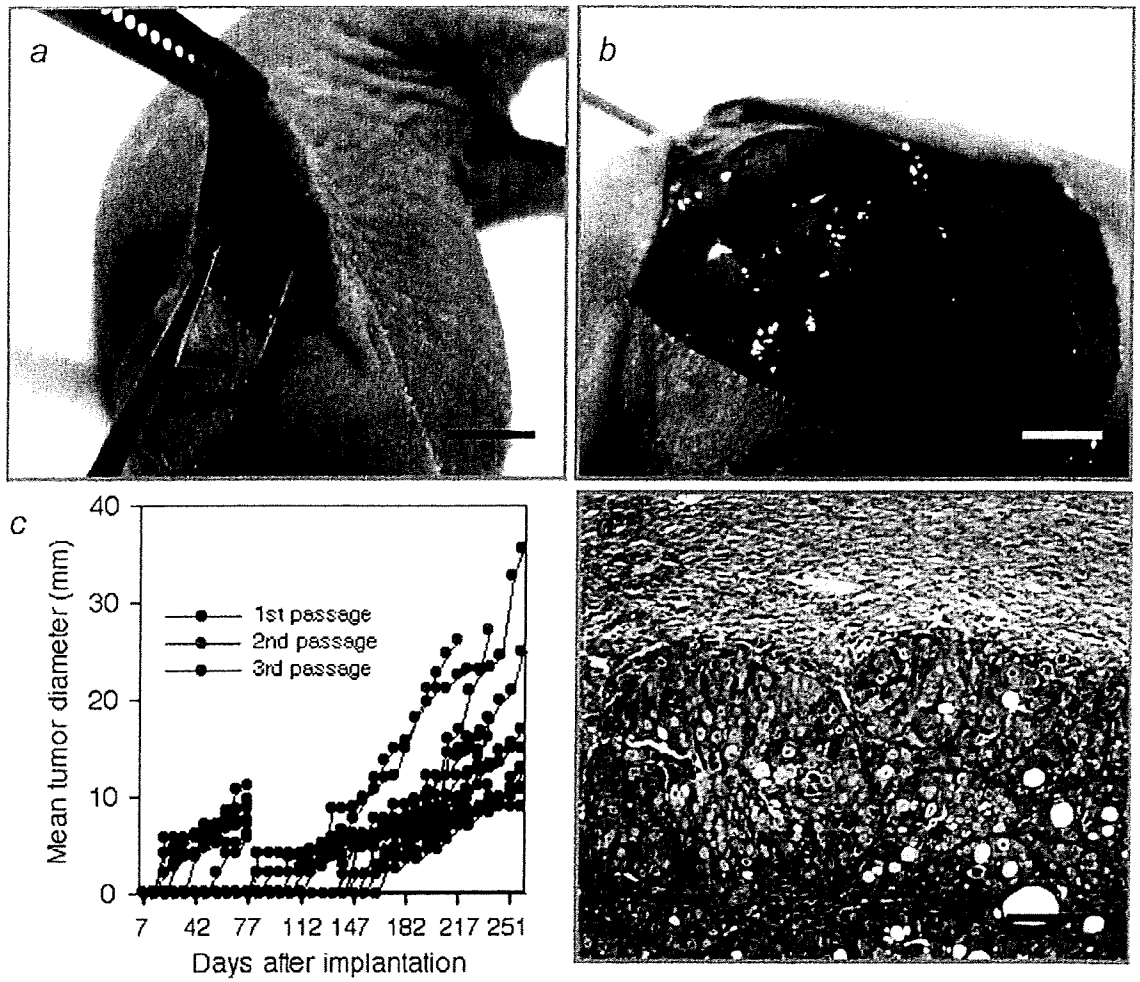

FIGURE 1 – Features of the arising adenocarcinoma after implantation of human colonic adenoma cells being attached to plastic plate. (*a*) Adenoma cells (1×10^5 cells per plate) were implanted into a subcutaneous space of nude mouse. Bar, 5 mm. (*b*) Around 5 months later, a palpable mass with forming angiogenesis appeared; the emergence was always monoclonal growth. Bar, 5 mm. (*c*) Latency period was shortened after serial transplantation of the arising adenocarcinoma cells with plastic plate attached. (*d*) Histologically the arising tumors were moderately differentiated adenocarcinoma with proliferating stroma (Azan staining). Bar, 100 μm.

transferred them into $gp91^{phox-/-}$ mice. As a result, wild-type-derived phagocytes increased the frequencies of tumor development and metastasis. In contrast, the phagocytes obtained from knockout mice did not have such activity.[98] Moreover, administration of aminoguanidine, a broad inhibitor for inducible nitric oxide synthase, partially but significantly suppressed malignant conversion in the model[93]; thus we concluded that RNS were also involved in the process. These results show that ROS and RNS, derived from foreign-body-induced neutrophils, are an intrinsic factor in the conversion of regressive tumors to more malignant ones. The mouse model may recapitulate the typical inflammation-based carcinogenesis, and thus be suitable for analyzing biological causes of the process. Gelatin sponge implantation induces massive and persistent infiltration of activated leucocytes; such situation may provide pathogenic resemblance to the continuous infiltration of activated inflammatory cells into target organs by bacteria (*Helicobacter pylori*[99]) or parasites (*Opistorchis* sp., *Chlonorchis* and *Schistosoma*[100]) infections in human.

Since we used rodent cells previously, we have been eager to prove the phenomena by using cells of human and epithelial origin. For this purpose, instead of establishing regressive tumors from lethally growing tumors, we have picked up an available cell line derived from precancerous tissues of colon. In patients with ulcerative colitis or Crohn's disease, a typical inflammation-related carcinogenesis is seen; that is, continuous infiltration of disordered immune cells into the autologous colon tissue precedes the development of colorectal cancer.[101,102] A culture cell line of adenoma cells (FPCK-1 cells) was established by Dr. Kawaguchi from a colonic polyp of a patient with familial adenomatous polyposis.[103] The phenotype of the cell line was stable and no spontaneous conversion was observed during the maintenance under regular cultivation at least for 1 and half years.

We used several sister lines of adenoma FPCK-1; they did not grow in nude mice when injected at 5×10^6 cells in a suspension form. However, adenoma cells of FPCK-1-1 formed palpable tumors in about 5 months when they were attached to plastic plate and implanted into subcutaneous space of mice (Fig. 1*a*).[104] Their tumorigenic growth always started from 1 colony (monoclonal origin) (Fig. 1*b*). Serial implantation of the culture cell line with plastic plate, established originally from the arising tumor after

TABLE I – UNIVERSALITY OF CARCINOGENESIS AND ITS PROGRESSION INDUCED BY FOREIGN BODIES IN THE CELLS DERIVED FROM IMMORTALIZED CELLS, REGRESSIVE TUMOR CELLS OR PRECANCEROUS LESIONS

Cells	Origin of the cell	Implantation host	Foreign body	Characteristics of arising tumors (reference)	Year
BALB/3T3	mouse, vascular endothelium	mouse	glass beads	malignant hemangioendothelioma (4)	1975
NCTC 8467	mouse lung tissue	mouse	glass helices	sarcoma (105)	1980
BALB/3T3	mouse, vascular endothelium	mouse	polycarbonate plastic plate	vasoformative sarcoma (5)	1976
C3H/10T1/2	mouse, embryo	mouse	polycarbonate plastic plate	fibrosarcoma (5)	1976
C3H/10T1/2	mouse, embryo	mouse	polycarbonate plastic plate	invasive fibrosarcoma (9)	1979
Connective tissues obtained from adult mice		mouse	polycarbonate plastic plate	undifferentiated sarcoma (8)	1979
ER-1	rat, regressive mammary adenocarcinoma	rat	polystyrene plastic plate	malignant, sarcomatoid histology (81)	1992
QR-32	mouse, regressive fibrosarcoma	mouse	polystyrene plastic plate	malignant fibrosarcoma (87)	1993
FPCK-1-1	human, colonic adenoma	mouse	polystyrene plastic plate	high tumor incidence, adenocarcinoma (104)	2000
Benzo(a)pyrene-induced sarcoma		mouse	polystyrene plastic plate	shortened latency period (112)	2002
Subcutaneous space of mice		p53 heterozygous mouse	polystyrene plastic plate	high tumor incidence, fibrosarcoma (72)	2007
Subcutaneous space of mice		p53 wild-type mouse	polystyrene plastic plate	low tumor incidence, fibrosarcoma (72)	2007
QR-32	mouse, regressive fibrosarcoma	mouse	gelatin sponge	malignant fibrosarcoma (88)	1992
Lk9dL	rat, nonmetastatic renal carcinoma	rat	gelatin sponge	metastatic renal carcinoma (107)	1998
FPCK-1-1	human, colonic adenoma	mouse	gelatin sponge	low tumor incidence, adenocarcinoma (104)	2000

implantation of the adenoma cells with plastic plate, shortened the latency period, in correlation with the number of passage (Fig. 1*c*).

Histologic examination revealed that the arising tumors were moderately differentiated adenocarcinoma, and they were surrounded by highly collagenic fibrous stroma (Fig. 1*d*). The fibrous tissue, rather than attachment to the plastic plate substrate, was considered essential for the conversion, because we observed tumor growth after injection of the adenoma cells directly at the site of proliferating stromal tissues where the plastic plate had been implanted for about 5 months and then removed. In contrast, there was no tumor development in nontreated or sham-operated mice, and tumor seldom arose after coimplantation with gelatin sponge in those, which indicated that acute inflammation did not suffice to convert the adenoma cells.[104] The stromas at the chronic inflammatory region secrete specific soluble factor(s), which have not been identified yet; we assume that the factor(s) could stimulate adenoma cell growth but not the growth of adenocarcinoma cells. Such factors could not be produced either from normal subcutaneous fibroblasts or immortalized mouse fibroblast cell lines.[104]

In our foreign-body-induced carcinogenesis and tumor progression models, we can observe that inflammation promotes malignancy of various species of cells. As summarized in Table I, the phenomenon is universally observed among species and in tissues of different origins.

Perspective of prevention of foreign-body-induced carcinogenesis

Inflammatory environments due to the existence of foreign body cause a variety of biological responses as they contain increased growth/survival factors, chemotactic cytokines (chemokines), matrix metalloproteases, adhesion molecules, extracellular matrix, inflammatory mediators (*i.e.*, histamine, eicosanoids, inflammatory cytokines and proteases), DNA-damage-promoting agents (*i.e.*, ROS and RNS) and augmented angiogenesis[108] (Fig. 2). I have been emphasizing the roles of inflammatory-cell-derived ROS in the foreign-body-induced carcinogenesis. In this section, I would like to suggest some strategies to prevent it. If the inflammatory-cell-derived ROS are critical to promote foreign-body-induced carcinogenesis, the sensitivity of cells to the conversion should be controlled by intracellular or tissue antioxidative potentials. Our earlier study demonstrated that a variation was observed among the QR clones in the frequency of tumor progression by coimplantation with foreign body.[96] We determined that the variation was due to intracellular antioxidative enzymes, manganese-superoxide dismutase, Mn-SOD and glutathione peroxidase, GPx level in the QR clone. An inverse correlation was observed between the contents/activities of those enzymes and the sensitivity of QR clones to progress under foreign-body-induced inflammatory environment.[96] Thus, we determined that cells with low antioxidative levels are prone to convert themselves into more malignant ones in the presence of inflammation and the inflammatory cell-derived ROS. In other words, foreign-body-induced carcinogenesis in QR cells will be prevented if the cells have an adequate amount of antioxidative enzymes or induction of the enzymes at the implantation site. The prevention was actually achieved by induction of Mn-SOD at the coimplantation site by administering Mn-SOD-inducible biological response modifier[109] or orally available superoxide dismutase in this system.[110] Moreover, it is assumed that RNS are partially involved in the model, because administration of a broad inhibitor for inducible nitric oxide significantly inhibited inflammation-induced tumor progression.[95]

By extensively analyzing human tissue materials obtained from typical inflammation-based carcinogenesis, it has been revealed that ROS and RNS are inevitably involved in the development and progression of tumors.[111,112] Considering those clinical studies

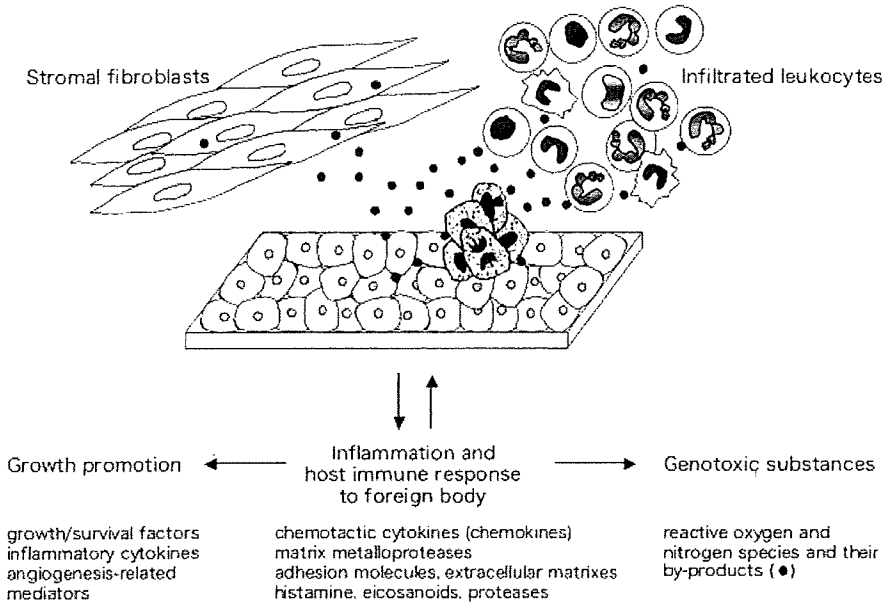

FIGURE 2 – Key biologic features of foreign-body-induced carcinogenesis. Malignant conversion of precancerous cells or progression of regressive tumor cells requires foreign body-induced inflammatory soluble mediators. The mediators can be classified as (*i*) growth promotion, (*ii*) inflammation and host immune response to foreign body and (*iii*) genotoxic substances (*i.e.*, reactive oxygen and nitrogen species). Each precancerous cell or regressive tumor cell possibly carries a variety of gene alterations. And only one of the cells may undergo the final crucial molecular changes that convert/progress to malignant one. Such clone-originated cell acquires various malignant phenotypes during proliferation under the influence of inflammatory soluble mediators.

and our results, we conclude that the proneness of tumor cells to be more malignant depends on the balance between antioxidative enzyme activities in themselves and the duration/amount of ROS and RNS generated by inflammatory cells, and that disturbing the balance will be the most effective strategy for the prevention of foreign-body-induced carcinogenesis.

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Malignant Fibrous Histiocytoma

Between the Past and the Present

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• The precise nature and diagnostic concept of malignant fibrous histiocytoma (MFH) has been debated for years. Currently, a histiocytic lineage of the tumor cells is no longer favored. The nomenclature and classification of MFH and its subtypes have also been changed. The MFH pattern, especially that of storiform-pleomorphic variant, is viewed as a morphologic pattern shared by a number of sarcomas as well as by other nonsarcomas. Therefore, a diagnosis of MFH based solely on morphology is no longer acceptable and identification of a line of differentiation should be sought. A diagnosis of MFH should be made only for pleomorphic sarcomas in which no specific line of differentiation is discerned. Precise categorization of MFH-like tumors may require thorough sampling of the tumor and judicious use of immunohistochemistry and/or electron microscopy. Familiarity with the current terminology and classification of MFH and its subtypes is of paramount significance in the modern practice of pathology.

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The term *malignant fibrous histiocytoma* (MFH) was first coined by Ozzello et al[1] in 1963 and described by O'Brien and Stout[2] in 1964. During the next 10 years, 5 variants of the tumor were introduced. The origin of the tumor cells has been a cause of an ongoing debate but it has become clear that the histiocyte is most likely not the cell of origin of the tumor.[3] Several studies have been published that cast doubt on the validity of MFH as a specific entity. In a major article published in 1992,[4] 74% of cases initially diagnosed as MFH were reclassified into other diagnoses after lines of differentiation were discovered on testing with more sensitive techniques. Malignant fibrous histiocytoma diagnosis was given only for the cases in which no line of differentiation could be determined. Since then, the integrity of MFH as a diagnostic entity has progressively diminished. At the present, there is a general consensus that the pattern of MFH, especially the storiform-pleomorphic subtype, represents a common morphology shared by many tumors and, therefore, every effort should be made to identify a line of differentiation before throwing the tumor in question into the wastebasket of MFH.[4] Because the fall of the "progenitor" should affect the "progeny," all MFH subtypes have also been reappraised and the terminology and classification of MFH and its subtypes have been reestablished in the latest World Health Organization (WHO) classification of soft tissue tumors.[5] In this article, we briefly review the current knowledge about MFH with regard to its histogenesis, most recent terminology and classification, and diagnostic approach.

THE PRESENT VIEW OF MFH HISTOGENESIS

Although the term *MFH* gives the impression that the tumor cells are of fibroblastic and histiocytic origin, the precise origin has been disputed for decades. With the advent of diagnostic techniques such as cell cultures, immunohistochemistry, and electron microscopy, a large number of studies attempted to elucidate the histogenesis of MFH.[6–8] The results of these studies are markedly conflicting, and often the disparity between the results is startling. Histiocytes, fibroblasts, or cells with features intermediate between fibroblasts and histiocytes, have all been proposed as the origin of the tumor cells.[9,10] A primitive mesenchymal stem cell that then manifested with both fibroblastic and histiocytic differentiation to varying degrees has also been suggested.[10] The bulk of evidence from all these studies suggests that MFH is a sarcoma of either fibroblastic or primitive mesenchymal origin, which manifests features of both fibroblastic and histiocytic differentiation. A true histiocytic origin is no longer acceptable.[11] Nevertheless, until the definitive origin of the tumor cells is reached, the 2002 WHO classification has maintained the tumor under the "fibrohistiocytic" category.[5]

The "Common Pattern/Ultimate Pathway" Hypothesis

Malignant fibrous histiocytoma is now viewed as a common "morphologic pattern" shared by a number of pleomorphic neoplasms, irrespective of their differentiation. Thus, MFH brings together varying tumors that can be actually unrelated but share similar morphologic features. Malignant fibrous histiocytoma is also thought to represent a "final common pathway" for tumor growth. As tumors (either sarcomatous or nonsarcomatous) progress in their growth, they may lose their differentiation pattern to reach an ultimate undifferentiated pattern, which is common to all of these tumors. These hypotheses

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are supported by a number of published studies that reevaluated tumors initially diagnosed as MFH. A specific line of differentiation (lipogenic, neurogenic, myogenic, or nonsarcomatous) was possible to ascertain in these cases. This may explain (1) the heterogeneity in immunophenotype, ultrastructure, and cytogenetics in many cases diagnosed as MFH; (2) the wide spectrum of clinical behavior (including treatment sensitivity, and time and pattern of metastasis), as well as the outcome of these cases; and (3) the frequent occurrence of MFH-like lesions in unusual locations. In a similar way, the morphologic characteristics of MFH subtypes are also found to be shared by a variety of other tumors, questioning their diagnostic entities and making it difficult to classify them under one category. Because of this view, the diagnosis of MFH and its subtypes became restricted to a small percentage of pleomorphic sarcomas in which all other possible lines of differentiation are excluded.[4,8]

NOMENCLATURE

The term *MFH* is perceived now as a misnomer because it points to a histiocytic origin of the tumor that is no longer tenable and it falsely includes unrelated tumors with a common morphology, as discussed earlier. Therefore, there are presently strong recommendations to abandon this term. *Pleomorphic sarcoma* is the alternate name advocated by the WHO to replace the current name as it gives a more accurate description of the tumor and does not imply the origin of the tumor cells.[12] The term *pleomorphic fibrosarcoma* has been suggested[13] but argued against because it may cause confusion with the classical fibrosarcomas, which are different entities consisting of a relatively uniform population of spindle cells almost always devoid of multinucleated or pleomorphic giant cells.[14] Unfortunately, the term *MFH* is deeply ingrained in surgical pathology and very familiar to surgeons and clinicians. Therefore, it is recommended that when the new nomenclature is used, the term *MFH* should be placed alongside the new term. In this manner, the WHO maintained the term *MFH* in its 2002 classification of soft tissue tumors.[5] Likewise, the same approach of tumor description appears in the American Joint Committee on Cancer 2002 staging system for soft tissue tumors.[15] Maintenance of the term *MFH* in the tumor description, at least for the time being, may provide an opportunity for this conceptual evolution to be widely used until the old term loses its appeal.

REAPPRAISAL OF MFH SUBTYPES

Five subtypes of MFH are present. These are (1) storiform-pleomorphic, (2) myxoid, (3) inflammatory, (4) giant cell, and (5) angiomatoid subtypes. It has now become clear that these subtypes are heterogeneous entities that should not be classified under a single category.

Storiform-Pleomorphic MFH

Before the collapse of MFH and its subtypes as diagnostic entities, the storiform-pleomorphic subtype of MFH had comprised the overwhelming majority of MFH cases in the literature (60%–70% of cases).[16] The storiform-pleomorphic MFH is typically composed of a mixture of spindle cells admixed with polygonal or rounded cells, arranged in a storiform pattern (Figure 1). A variable number of bizarre, multinucleated giant cells are also present. Marked cellularity and nuclear pleomorphism with abundant atypical mitoses are usually evident in this high-grade tumor (Figure 2). Storiform-pleomorphic MFH is usually found on the lower limbs of elderly patients (sixth and seventh decade). The retroperitoneum is also a common location. The aforementioned common pattern/ultimate pathway hypothesis is clearly represented in the storiform-pleomorphic MFH, in which the tumor morphology is shared by a number of undifferentiated tumors including sarcomas as well as nonsarcomatous tumors such as undifferentiated carcinomas (Figure 3), spindle cell melanomas, and spindled lymphomas. Therefore, the diagnosis of storiform-pleomorphic MFH is one of exclusion when no line of differentiation is identified. In such cases, an alternate name of *undifferentiated high-grade pleomorphic sarcoma* is being advocated by the WHO in its 2002 classification of soft tissue tumors. The tumor is still classified under the category of fibrohistiocytic tumors.[5] After this conceptual shift, this subtype, which was once considered the most common soft tissue tumor in adults, now accounts for no more than 5% of adult soft tissue tumors.

Myxoid MFH

Myxoid MFH represented the next most common subtype of MFH (10%–20% of cases). Microscopically, the tumor shows prominent myxoid matrix. Myxoid MFH is now seen as a specific entity because not only does it have a distinctive myxoid appearance (Figure 4) but it has also been shown to have a better prognosis than other subtypes of MFH. Therefore, the 2002 WHO classification uses the term *myxofibrosarcoma* for this tumor.[5] As myxofibrosarcoma displays myogenic differentiation (shows immunoreactivity to smooth muscle or muscle-specific actin),[17] myxofibrosarcoma has been removed from the fibrohistiocytic category and reallocated to the myofibroblastic one.[5]

Giant Cell MFH

Giant cell MFH was a rare variant (10%–15% of cases). The tumor is microscopically characterized by multinucleated giant cells. The giant cells closely resemble osteoclasts but their nuclei tend to be higher grade and are not usually found in association with osteoid. It is now appreciated that giant cell MFH does not represent a specific entity as it is usually possible to identify a line of differentiation in the majority of cases. Many cases initially diagnosed as giant cell MFH have been reclassified as giant cell–rich osteosarcoma, leiomyosarcoma with an osteoclastic giant cell reaction, or giant cell–rich anaplastic carcinoma.[18] In cases in which no evidence of differentiation is found, diagnosis of giant cell MFH can be made, using its new terminology, which is *undifferentiated pleomorphic sarcoma with giant cells.* The tumor is classified under the category of fibrohistiocytic tumors in the 2002 WHO classification.[5]

Inflammatory MFH

Inflammatory MFH was the rarest variant (5% of cases). The tumor characteristically shows an intense inflammatory infiltrate that consists predominantly of neutrophils, lymphocytes, and foamy histiocytes (Figure 5). Similar to the other MFH variants, the entity of this variant has been questioned. Many cases initially diagnosed as inflammatory MFH were recognized to be dedifferentiated liposarcomas, in which the dedifferentiated component has a prominent stromal inflammatory infiltrate. Other tumors that closely mimic inflammatory MFH include anaplastic

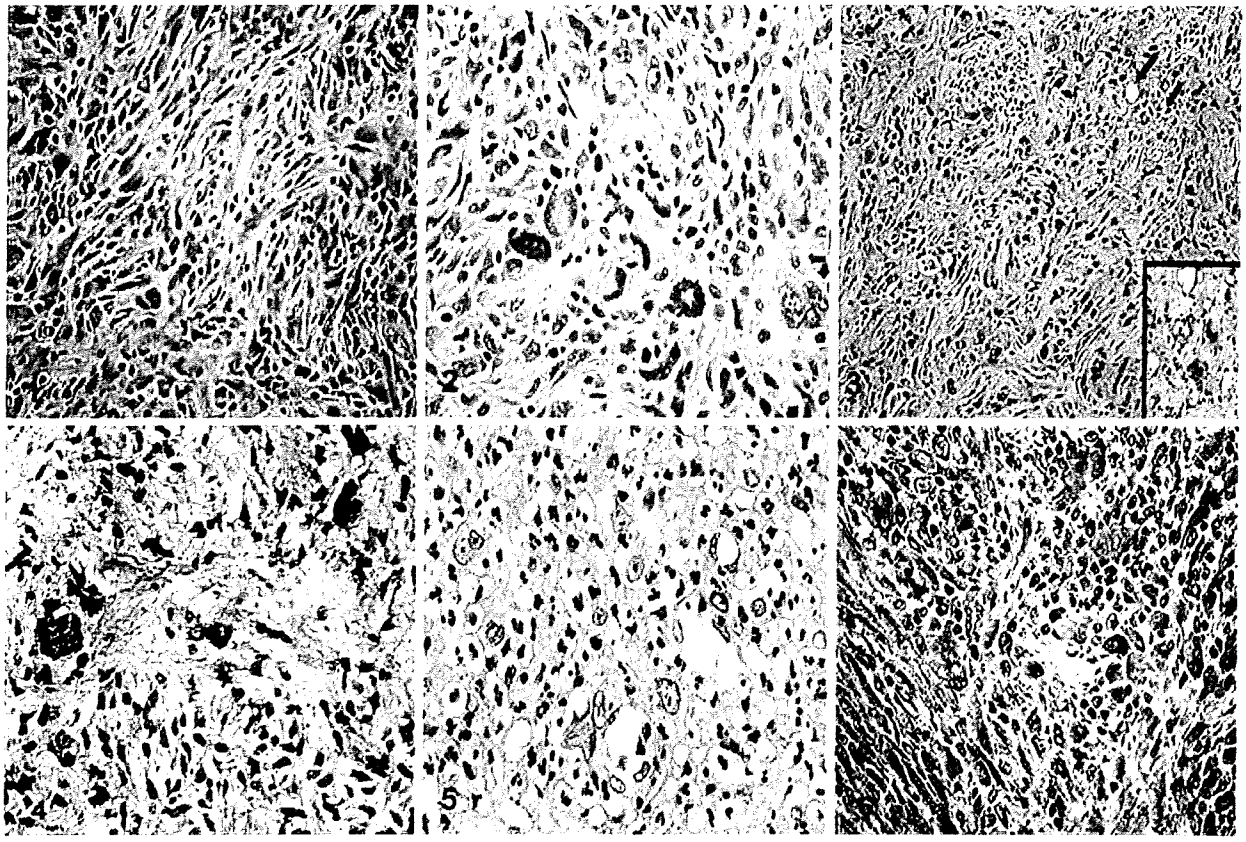

Figure 1. *Storiform-pleomorphic malignant fibrous histiocytoma/undifferentiated high-grade pleomorphic sarcoma. Exclusion of all lines of differentiation is necessary before making such diagnosis (hematoxylin-eosin, original magnification ×150).*

Figure 2. *The cells of malignant fibrous histiocytoma show frank malignant features: marked cellular atypia with bizarre multinucleated giant cells and brisk atypical mitotic figures reflecting the high grade of the tumor (hematoxylin-eosin, original magnification ×400).*

Figure 3. *Poorly differentiated adenocarcinoma with areas indistinguishable from the pattern of storiform-pleomorphic malignant fibrous histiocytoma (MFH). Careful examination of the tumor shows differentiated areas in the form of mucin-laden vacuoles (arrows) stained positive with mucicarmine (inset). These differentiated areas appeared on extensive sampling of the tumor. This case is an example of the common pattern hypothesis. The extensive sampling in this case helped reveal the line of differentiation and avoid erroneous diagnosis of MFH (hematoxylin-eosin, original magnification ×150, and mucicarmine, original magnification ×300 [inset]).*

Figure 4. *Myxoid malignant fibrous histiocytoma/myxofibrosarcoma showing pleomorphic nuclei in a myxoid matrix (hematoxylin-eosin, original magnification ×150).*

Figure 5. *Inflammatory malignant fibrous histiocytoma/undifferentiated pleomorphic sarcoma with prominent inflammation showing an inflammatory infiltrate composed mainly of lymphocytes, plasma cells, and neutrophils admixed with cytologically malignant pleomorphic xanthomatous and giant cells. Exclusion of all lines of differentiation is necessary before rendering such diagnosis (hematoxylin-eosin, original magnification ×150).*

Figure 6. *The tumor cells of storiform-pleomorphic malignant fibrous histiocytoma (MFH)/undifferentiated high-grade pleomorphic sarcoma show diffuse and strong cytoplasmic immunoreactivity to vimentin. Other immunostains fail to discern any line of differentiation. This "vimentin only" immunophenotype should lead to the diagnosis of MFH (original magnification ×150).*

carcinomas with prominent inflammation and anaplastic large cell lymphomas.[19] Therefore, the diagnosis of inflammatory MFH can be made only if no line of differentiation is identified. In the 2002 WHO classification, inflammatory MFH has been renamed as *undifferentiated pleomorphic sarcoma with prominent inflammation* and classified under the category of fibrohistiocytic tumors.[5]

Angiomatoid MFH

Angiomatoid MFH is now no longer considered as an MFH subtype. This is because the tumor is morphologically and clinically distinct from MFH and its variants. The tumor occurs predominantly in children and young adults. Microscopically, the tumor is benign-looking with eosinophilic, oval, round, or spindled cells with slight pleomorphism, arranged in sheets and whorls. There are prominent slitlike vascular channels with an inflammatory infiltrate and focal areas of hemosiderin deposits and hemorrhage. Moreover, the tumor tends to run an indolent course with very infrequent metastasis. The benign nature of the tumor is reflected by its new name: *angiomatoid fibrous histiocytoma*. Although a myogenic differentiation of the tumor was evidenced by its desmin immunopositivity in about half of the cases, the precise line of differentiation is still unknown as concurrent epithelial differentiation is also frequently encountered (evidenced by its immunore-

Nomenclature and Categorization of Malignant Fibrous Histiocytoma (MFH) Subtypes (2002 World Health Organization Classification)		
Old Nomenclature of MFH Subtype	Current Nomenclature of MFH Subtype	Tumor Category
Storiform-pleomorphic MFH	Undifferentiated high-grade pleomorphic sarcoma	Fibrohistiocytic
Myxoid MFH	Myxofibrosarcoma	Myofibroblastic
Giant cell MFH	Undifferentiated pleomorphic sarcoma with giant cells	Fibrohistiocytic
Inflammatory MFH	Undifferentiated pleomorphic sarcoma with prominent inflammation	Fibrohistiocytic
Angiomatoid MFH	Angiomatoid fibrous histiocytoma	Tumors of uncertain differentiation

activity to epithelial membrane antigen).[20] Therefore, angiomatoid fibrous histiocytoma was removed from the category of fibrohistiocytic tumors in the 2002 WHO classification and reallocated to the category of tumors of uncertain differentiation.[5]

The Table lists the nomenclature and classification of MFH subtypes.

DIAGNOSTIC APPROACH TO MFH-LIKE TUMORS

Although it is emphasized that efforts should be expended to identify a line of differentiation in all MFH-like lesions, no published guidelines to define a diagnostic approach are available. However, careful and/or extensive sampling with the use of ancillary techniques, immunohistochemistry in particular, should be 2 important aspects of any diagnostic process.

Careful and/or Extensive Sampling

The diagnostic approach of any tumor resembling MFH can be initiated by careful sampling of the tumor in quest for areas that may help identify a line of differentiation. If the initial sections are not helpful in identifying a line of differentiation, additional sampling can help reveal more differentiated areas or at least some diagnostic features that were not present in the initial sections. In a published study,[21] 3 retroperitoneal lesions of dedifferentiated liposarcoma were initially misdiagnosed as MFH. This was because of lack of sufficient sampling as only 2, 3, and 1 paraffin blocks were performed on the 3 cases and none showed the well-differentiated liposarcoma component. The recurrent tumors were sampled extensively (37, 58, and 40 paraffin blocks). Areas of well-differentiated liposarcoma were seen on few slides, and in 1 case the well-differentiated liposarcoma component was seen on 1 slide only. Careful and extensive sampling was substantial in these cases to identify a line of differentiation and thus avoiding the erroneous diagnosis of MFH.

Immunohistochemistry

The use of immunohistochemistry is now essential in the diagnostic workup of any MFH-like tumor as it is now unacceptable to diagnose MFH based on morphology alone. Because the diagnosis of MFH is one of exclusion, an extensive immunohistochemistry panel is most likely required to exclude different lines of differentiation. There are no clear guidelines as to how extensive the panel of immunomarkers should be, but judicious use of immunomarkers should always be sought.[12] The panel can start with immunomarkers of broad differentiation such as vimentin and cytokeratins for mesenchymal and epithelial differentiation, respectively. The panel can then be narrowed with more specific markers that can be added according to the clinical setting and histologic suspicion. Undifferentiated pleomorphic sarcoma typically demonstrates immunoreactivity to vimentin but fails to show reactivity to immunostains of other lines of differentiation (Figure 6). Contrary to what is traditionally known, histiocytic markers (CD68, α_1-antitrypsin, α_1-antichymotrypsin, and factor XIII) no longer play a useful role in the diagnosis of MFH as immunoreactivity to these markers is found to be nonspecific and, therefore, will not support a definitive diagnosis of MFH.[22–24]

Electron Microscopy

When the histomorphology and immunophenotype of the tumor are not distinctive enough for a specific diagnosis, electron microscopy can be an additional technique in searching for a line of differentiation in MFH-like tumors. Electron microscopy can provide answers when immunohistochemistry does not identify the tumor, especially when a tumor expresses small foci of apparently aberrant marker. For example, focal expression of cytokeratin or the presence of scattered smooth muscle actin–positive cells is not infrequent in undifferentiated pleomorphic sarcomas and should not be taken as evidence of carcinomatous or myogenic differentiation, respectively. In such cases, electron microscopy may confirm the differentiation line through finding the characteristic ultrastructural features.[25]

Molecular Techniques

Numerous studies have reported different genetic abnormalities in MFH, but conclusive cytogenetic data are not yet available, thus limiting the usefulness of molecular procedures in the diagnostic workup of MFH, at least for now.[26]

Until more ancillary studies are introduced, careful sampling and immunohistochemical studies are usually recommended toward obtaining the definitive diagnosis of MFH. Nevertheless, this diagnostic approach has some limitations. It is based on the assumption that significant amounts of tissue are available for extensive sampling. This may not be true, especially when core needle biopsies, which limit the amount of tissue available, are used. Moreover, the diagnostic workup can be exhaustive and costly if extensive sampling and a large panel of immunostains are required. Therefore, surgical pathologists have to pursue the most accurate and cost-effective interpretations within the constraints of time and cost. It is necessary that accurate and reproducible guidelines be drawn up to aid in logical and orderly search for lines of differentiation in MFH-like tumors.

SIGNIFICANCE OF SEARCHING FOR DIFFERENTIATION IN MFH-LIKE TUMORS

Searching for differentiation in MFH-like tumors can be clinically significant for therapeutic and prognostic reasons.

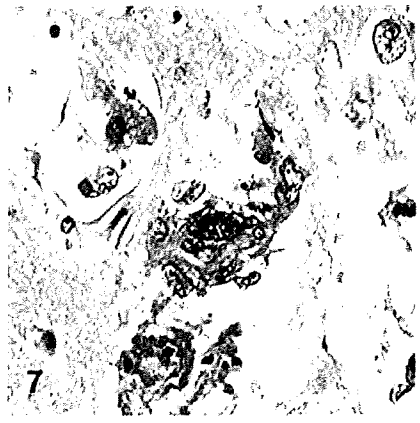

Figure 7. *Bizarre multinucleated giant cells without specific cytomorphologic differentiation are frequently seen in the aspirate of malignant fibrous histiocytoma, storiform-pleomorphic subtype (Papanicolaou stain, original magnification ×150).*

Therapeutic Significance

Because the MFH morphologic pattern is shared by many tumors of different lineages, identification of differentiation is of therapeutic significance because the tumors differ in the treatment modality. For example, poorly differentiated adenocarcinomas, spindled lymphomas, and melanomas can have MFH-like patterns but their treatment is different. If a diagnosis of MFH is erroneously given in such cases based on morphology only, inappropriate therapy would be instigated.

Prognostic Significance

Identification of subsets of pleomorphic sarcomas can be of a prognostic significance. For example, pleomorphic sarcomas with myogenic differentiation such as pleomorphic rhabdomyosarcoma usually show more aggressive behavior than pleomorphic sarcomas with nonmyogenic differentiation such as pleomorphic liposarcoma.[27,28]

CYTOLOGY

Fine-needle aspiration of MFH lesions is usually of limited value in the differential diagnosis. The cytologic features of MFH on fine-needle aspirations are not specific and include clusters of atypical polygonal and spindle cells with bizarre, multinucleated giant cells (Figure 7). The neoplastic cells do not show cytomorphologic evidence of any specific tissue differentiation. Because an extensive immunohistochemical panel is usually required in the diagnostic workup of MFH-like tumors, it is unreasonable to expect a precise classification of these tumors based solely on fine-needle aspiration,[29] especially if the cytology cases do not have enough cell block material to perform the immunocytochemistry. Therefore, it is better to avoid the diagnosis of MFH based on cytologic appearance alone. However, diligent search for cells or features that may help demonstrate specific differentiation (lipoblasts, rhabdomyoblasts, keratinization, and mucin production) should be attempted as this helps exclude the diagnosis of MFH.

Lastly, we have to point out that the shift in the diagnostic criteria of MFH has affected the literature. This change may affect the credibility of many reported MFH cases. Pathologists should exercise caution in accepting a diagnosis of MFH as valid if the diagnosis is made on the basis of morphology alone, especially in the era before the introduction of immunohistochemistry. Moreover, the published clinical trials regarding the therapeutic modalities and prognosis of MFH should be updated with new therapeutic strategies restricted to the unclassifiable group of tumors—the pleomorphic sarcomas.

CONCLUSIONS

Malignant fibrous histiocytoma is a malignant soft tissue tumor composed of tumor cells without evidence of a specific tissue differentiation. It is now widely accepted that the so-called MFH is not a diagnostic entity as it represents a common, final pathway of many sarcomatous as well as nonsarcomatous tumors. Its features are shared by a variety of poorly differentiated malignant neoplasms. The term *MFH* is now reserved for the small group of truly undifferentiated pleomorphic sarcomas. In any MFH-like tumor, a line of differentiation should be searched for, leaving the unclassifiable or difficult-to-categorize lesions in the category of the formerly known MFH. If a line of differentiation is identified, the diagnosis should be made based on that specific differentiation. Careful sampling with the use of immunohistochemistry should prevent overdiagnosis of MFH. The tumor cells of MFH typically show a "vimentin only" immunophenotype, that is, diffuse immunoreactivity to vimentin with failure of other immunostains to discern any line of differentiation. The current classification and nomenclature of the entity formerly known MFH should be referred to while reporting the diagnosis.

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