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MFH classification: differentiating undifferentiated pleomorphic sarcoma in the 21st century

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Abstract

The essence and origin of malignant fibrous histiocytoma (MFH) have been debated for now close to five decades. Originally characterized as a morphologically unique soft tissue sarcoma subtype in 1963 of unclear etiology with a following decade and a half of research only to conclude that “the issue of histogenesis [of MFH] is largely unresolvable”; it is “now regarded as synonymous with [high grade] undifferentiated pleomorphic sarcoma [HGUPS] and essentially represents a diagnosis of exclusion”. Yet despite this apparent lack of progress, the first decade of the 21st century has seen some significant progress in terms of defining the origins of MFH. Perhaps more importantly these origins might also pave the way for novel therapies. This manuscript will highlight MFH’s troubled history, discuss recent advances, comment as to what the coming years may promise, and what further needs to be done to make sure that progress continues.

Keywords

Malignant fibrous histiocytoma (MFH); undifferentiated pleomorphic sarcomas; mesenchymal stem cells; differentiation therapy; classification

Classification of Sarcomas

There are approximately 12,000 new adult sarcoma cases each year [1], encompassing seventy different histologic types of mesenchymal tumors that arise from bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. An in depth understanding of the molecular biology for most of these tumor types is unfortunately lacking [2] thus greatly hindering our abilities to develop rational therapeutic options.

Cytogenetics has long been used to divide sarcomas into two broad groups [3]: (1) sarcomas with specific genetic alterations and usually simple karyotypes, including reciprocal translocations that result in fusion genes; and (2) sarcomas with nonspecific genetic

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alterations and complex unbalanced karyotypes, evidenced by numerous chromosomal losses and gains. Tumor progression in both simple and complex karyotype tumors are commonly associated with additional mutations in known cell cycle regulatory pathways (Rb, p53, and/or their upstream/downstream targets; reviewed in [2]). Sarcomas with simple karyotypes have characteristic chromosomal translocations that produce specific gene fusion products, pathognomonic for a certain sarcoma subtype. To date, 41 gene fusions in 17 subtypes of sarcomas have been identified [4]. These fusion products encode for either transcription factors (e.g., Ewing sarcoma [5]) or growth factors (e.g., dermatofibrosarcoma protuberans [6]). Generally, these are each thought to deregulate the expression of specific repertoires of downstream target genes, possibly providing multiple oncogenic hits analogous to the multistep process of epithelial carcinogenesis. So far, secondary mutations have been identified in many of these sarcomas [7], but none have been shown to be necessary for tumorigenesis. Sarcomas with complex karyotypes are characterized by extensive cytogenetic abnormalities (e.g., dedifferentiated liposarcoma, leiomyosarcoma). These include chromosomal deletions, amplifications, gains and losses of whole chromosomes, and aneuploidy [8]. Although there is an association between cumulative chromosomal abnormalities and high-grade tumors [3], there does not seem to be a consistency to the genetic damage within a tumor subtype. The finding of multiple and complex genetic abnormalities in these sarcoma subtypes makes identifying the contribution of any one abnormality difficult. It is this latter issue that has relegated cytogenetics as an adjunct to histopathology in the diagnosis and classification of sarcomas.

Sarcoma histopathology classification has itself proven to be difficult, since in addition to the great histological heterogeneity, the problem in studying sarcomas is further compounded by the fact that numerous histological sub-types exist without a known site or tissue of origin. These subtypes include, but are not limited to, malignant fibrous histiocytomas (*MFH*), Ewing's sarcoma, alveolar soft parts sarcoma (ASPS), desmoplastic small round cell tumor, and epithelioid sarcoma. Note that for carcinomas there are no distinct histological sub-types of carcinomas of unknown primary which account for approximately 3–5% of all metastatic carcinomas at presentations [9,10]; while for sarcomas there are numerous sub-types indicating distinct entities without a known tissue or cell of origin. Furthermore, these sarcoma sub-types of unknown origin may account for up to one-third of all sarcoma patients. *MFH* alone have historically accounted for approximately 25% of all patients accrued to sarcoma clinical trials [11,12].

MFH: Origins and Expansion

The origin of the term “malignant fibrous histiocytoma (MFH)” dates back almost fifty years to the early 1960s and our own institutional predecessors working at Columbia University. Margaret R. Murray observed that cells from cultured soft tissue sarcomas were characterized by a storiform (i.e., cartwheel-like growth) pattern, with pleomorphic and giant tumor cells which seemed to display ameba-like movement as well as phagocytosis. These features seemed reminiscent of histiocytes (i.e., local resident tissue macrophages). Upon further growth, these cells were capable of elongating and assuming a fibroblastic appearing pattern. Based on these observations, Murray in conjunction with Arthur Purdy Stout postulated that pleomorphic soft tissue tumors arose from histiocytes capable of fibroblastic transformation, the so-called “facultative fibroblast” [13]**. The term ‘malignant fibrous histiocytoma’ seemed appropriate and entered the literature.

The next two decades confirmed and expanded the diagnostic entity of MFH [14–17]. Since the neoplasms designated as MFH continued to exhibit a wider and wider range in terms of histological appearances during this time, they were further subdivided into 5 types: (1)

storiform-pleomorphic, (2) myxoid (myxofibrosarcoma), (3) giant cell (malignant giant cell tumor of soft parts), (4) inflammatory, and (5) angiomatoid (reviewed thoroughly in [18]*).

Storiform-Pleomorphic MFH

The storiform-pleomorphic subtype of MFH had historically comprised the majority of MFH cases, accounting for up to 70% of all reported cases. It is typically composed of a mixture of spindle cells and polygonal or rounded cells, arranged in a storiform pattern. The tumor is usually high grade containing additional bizarre, multinucleated giant cells as well as a marked cellularity, nuclear pleomorphism, and abundant atypical mitoses.

Myxoid MFH

Myxoid MFH represented the next most common subtype of MFH (10%–20% of cases). Microscopically, the tumor tends to show a prominent vacuolated matrix.

Inflammatory MFH

Inflammatory MFH was the rarest variant (5% of cases). The tumor characteristically shows an intense inflammatory infiltrate consisting of neutrophils, lymphocytes, and foamy histiocytes.

Giant Cell MFH

Giant cell MFH accounted for 10%–15% of cases. The tumor is defined by the presence of multinucleated giant cells. These giant cells resemble osteoclasts but are high grade in their nuclear features and are not associated with osteoid matrix.

Angiomatoid MFH

The tumor occurs predominantly in children and young adults. This tumor is microscopically benign-looking with eosinophilic, oval to round, or spindled cells with slight pleomorphism, arranged in sheets and whorls.

Due to the inclusive nature of the diagnosis malignant fibrous histiocytoma thus became the most common type of soft tissue sarcoma prescribed to adults until the 1990s.

MFH: Contraction/Retraction

Perhaps it was the growing number of cases that were being classified as MFH during the mid 1980s that began to cause pathologists concern. Some pathologists felt that the MFH diagnosis was being overused, and that it had become nothing more than a diagnosis of exclusion [18]*. Other pathologists, however, felt that MFH was not a diagnosis that was to be alternatively over or under used – but more of a histopathological or morphologic pattern that may be common to many sarcomas [19]. In a pattern that would repeat itself every ten years or so, a large group of previously categorized MFH tumors were re-reviewed using the then most modern immunohistochemical and electron microscopic techniques available in 1992. Only 13% percent were re-diagnosed as MFH [20]. A similar re-review in 2002 by a different group would yield only a 27% concordance rate [21].

This lack of consistency has led most pathologists to endorse the blanket statement that precise classification of pleomorphic sarcoma/MFH requires thorough sampling of a tumor and appropriate use of immunohistochemistry and electron microscopy [22]**.

As of the 2002 WHO classification much of what was previously considered to be MFH has been revamped [22–26]**.

Myxoid MFH

This tumor is no longer subtype of MFH. The 2002 WHO classification uses the term myxofibrosarcoma for ‘myxoid’ MFH. As myxofibrosarcoma displays myogenic differentiation (shows immunoreactivity to smooth muscle or muscle-specific actin), myxofibrosarcoma has been removed from the fibrohistiocytic category and reallocated to the myofibroblastic list.

Angiomatoid MFH

This tumor is no longer a subtype of MFH. It has been renamed angiomatoid fibrous histiocytoma. This tumor tends to be indolent with rare metastasis. The precise lineage has been recently questioned as this tumor displays both desmin and epithelial membrane antigen and it is now classified under the category of tumors of uncertain differentiation.

Giant Cell MFH

Giant cell MFH does not represent a specific entity, as it is possible to identify a line of differentiation in the majority of cases. Many cases initially diagnosed as giant cell MFH have been subsequently reclassified as giant cell – rich osteosarcoma, leiomyosarcoma with an osteoclastic giant cell reaction, or giant cell – rich anaplastic carcinoma. 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.

Inflammatory MFH

Similar to the other MFH variants, the existence of inflammatory MFH as a distinct entity has also been questioned. Many cases that were initially diagnosed as inflammatory MFH were subsequently reclassified as a dedifferentiated liposarcomas with a prominent stromal inflammatory infiltrate. The diagnosis of inflammatory MFH can be made only if all markers of a mesenchymal lineage are negative. In the 2002 WHO classification, inflammatory MFH has been renamed as undifferentiated pleomorphic sarcoma with prominent inflammation.

Pleomorphic MFH

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.

MFH Common Usage and Perception – Present Day

Thus as of 2002, the term “undifferentiated pleomorphic sarcoma”, either without modification or further modified by giant cell or inflammation, has replaced the old MFH terminology. With this conceptual shift, the relocation of the myxoid and angiomatoid MFH to other categories, and more rigid 21st century criteria; MFH, which once were considered the most common soft tissue neoplasms in adults, now account for no more than 5% of adult soft tissue sarcomas [26].

There are currently two schools of thought regarding the etiology of MFH/undifferentiated pleomorphic sarcomas. The more common is that these tumors do not actually represent a type of cancer but rather a common “morphologic pattern” shared by many neoplasms, irrespective of their differentiation. Thus, MFH brings together varying tumors that can be actually unrelated but share similar morphologic features. This common morphologic pattern can be the result of a final common pathway of cancer progression. In this model it is

presumed that tumors become progressively more undifferentiated, ultimately resulting in a high grade undifferentiated pleomorphic sarcoma. Interestingly enough this can happen not only in sarcomas but also carcinomas as well [22,23,25].

The second school of thought postulates that undifferentiated sarcomas are not the result of loss of differentiation markers from previously differentiated sarcomas or carcinomas; but rather they are the results of transformation of mesenchymal stem cells. This model is very reminiscent to that of “embryonal carcinoma”. The latter is composed of undifferentiated cells of epithelial appearance, with abundant clear-to-granular cytoplasm, and a variety of growth patterns. In pure form, embryonal carcinoma comprises only 2–10% of all germ cell tumors; while it is described as a component in more than 80% of testicular germ cell tumors [27,28]. While the heterogeneity of sarcomas make it more difficult to clearly define isolated patterns characteristic of a certain type within the same tumor mass; different parts of the same tumor may represent that tumor at different stages of differentiation [22,23,25].

For nearly two decades [29], cancer cells of solid tumor malignancies, including sarcomas, have been considered to be derived from well-differentiated normal cells, such as mature connective tissue, via multiple incidents of damage to their genome, resulting in activation of oncogenes or inactivation of tumor suppressor genes, which accelerate proliferation and accentuate the malignant phenotypes. Specifically with regards to sarcomas, this hypothesis of multi-step carcinogenesis states that well differentiated sarcomas (e.g., well differentiated liposarcomas) arise from normal fat tissue, which upon further genetic alterations form the more aggressive and less differentiated corresponding sarcomas (i.e., dedifferentiated liposarcoma). In contrast, a differentiation based model of solid carcinogenesis, first proposed three and a half decades ago [30], states that histological sub-types of a given malignancy result from the transformation of differentiating cells at various time points during normal differentiation. According to this model, transformation at early points of connective tissue differentiation would result in the less differentiated sarcomas corresponding to differentiating lineage, while transformation at later time points of differentiation would result in the well differentiated sarcomas. Both models have their strengths and weaknesses.

Linking Undifferentiated Sarcomas to Mesenchymal Stem Cells

In our opinion, a major obstacle in the study of the origins of solid tumors has been the lack of identified distinct stages of normal cellular maturation. For hematopoietic systems, the identification of hematopoietic stem cells and characterization of cell surface markers corresponding to distinct stages of hematopoietic-lymphoid development was the first line of evidence suggesting that there may be a relationship between these stages and specific lymphoid malignancies [31]. For solid tumors, mature tissue cells was all that was available for comparison to tumors until relatively recently [32]. In the absence of defined stages of cellular maturation, the only conclusion possible was that tumors were either more or less similar to (i.e., well-differentiated or undifferentiated) to their corresponding mature tissue. No information was available about the relationship between undifferentiated tumors and early differentiating tissues/cells. In fact, there is no mention of a comparison to normal development in any of the World Health Organization (WHO) texts on solid tumor classification. While in the hematopoietic system, it could be stated with significant certainty that Burkitt’s lymphoma morphologically and via cell surface antigen analysis resembles a follicular B-cell lymphoblast while small cell lymphoma resembles naïve B-cells. This kind of analysis was, and still mostly is, not possible for solid tumors.

The mesenchymal stem cell (*MSC*) was the first solid tissue stem cell isolated and is by far the best characterized [32]. MSCs were first described and isolated in 1980 [33] as adherent fibroblast like appearing cells within bone marrow aspirates capable of forming self-

sustaining colonies, and differentiating into multiple mesenchymal lineages under appropriate culture conditions. Since then, MSCs have been isolated in mice, rats, pigs, and rabbits (reviewed in [34]). While surface marker/antigen profiling of MSCs has been extensively performed (as for hematopoietic stem cells) the results have been varied (reviewed in [34]). More recently, the International Society for Cellular Therapy (ISCT) attempted to standardize the mesenchymal stem cell (MSC) field by defining MSCs as: 1) plastic-adherent under standard culture conditions; 2) expressing CD105, CD73 and CD90, and lacking expression of CD45, CD34, CD14 or CD11b, CD79 or CD19 and HLA-DR; and 3) being able to differentiate into osteoblasts, adipocytes and chondroblasts *in vitro*. Historically many MSCs isolated met criteria 1 and 3, but cell surface profiling was not always done, and even if it was, there was little consensus among groups regarding marker selection.

Following the characterization of mesenchymal stem cells two types of cells or maturation stages came into existence with which to compare and contrast sarcomas: the mesenchymal stem cell and the mature connective tissue/cell. To the best of our knowledge, Gazziola et al [35]** were the first to attempt to do such a comparison. Using a differential display-based gene expression platform, they profiled and compared bone-marrow derived MSCs with primary undifferentiated sarcomas; leiomyosarcoma with smooth muscle cells, and fibrosarcoma with fibroblasts. They were able to demonstrate that there was as much similarity between MSCs and undifferentiated sarcomas as there was for leiomyosarcomas and smooth muscle cells, and for fibrosarcomas and fibroblasts.

Building on the tumorigenesis-differentiation theme, our group further hypothesized that different sub-types of sarcomas within the same lineage may be representative of transformation at different time-points during the differentiation of mesenchymal cells into that mature lineage. We isolated total RNA at multiple time points during the 21-day *in vitro* adipocytic or osteoblastic differentiation program of hMSCs [36], performed gene expression analysis using the U133a Affymetrix array, identified an adipocytic differentiation gene set, and used those genes to perform distance analysis mapping against the gene expression analysis profiles of the four major subtypes of liposarcomas as determined by our previous gene expression profiling [37]. Our results showed that dedifferentiated LS correlated best to day 7 adipocytic-differentiating cells, pleomorphic LS to day 10, myxoid and round LS to day 14, and well-differentiated LS to day 21 adipocytic-differentiating cells [38].

Additionally, we isolated total cellular RNA from an undifferentiated pleomorphic sarcoma (MFH22), a liposarcoma cell line (LS141) and the SAOS2 osteosarcoma cell line. Performing hierarchical clustering using the adipocytic-specific gene set demonstrated that in the adipocytic series, the MFH22 cell line associated with MSC, while the LS141 cell line associates with later time points (i.e., cells committed to the adipocytic lineage). Similarly, performing hierarchical clustering using the osteogenic gene set demonstrated that in the osteogenic series, the MFH22 cell also associates with the MSCs, while the SAOS2 cell line associates with later time points (i.e., cells committed to the osteogenic lineage) [39]**. This set of differentiation experiments in cell lines independently confirms the association studies described on the human sarcoma samples.

To determine whether the gene expression profiles of high grade undifferentiated sarcomas supported our growing data that MFH was derived from MSCs, RNA was isolated from proliferating MSCs, analyzed on Affymetrix U133a microarrays, and compared to sarcomas that our laboratory had previously profiled [37]. We used a well-characterized panel of stem cell-specific genes [40,41] to evaluate the potential relationship between MSCs, undifferentiated sarcomas, and other sarcoma subtypes. Unsupervised hierarchical clustering

analysis using such a “stem cell gene signature” revealed that MSCs are significantly and uniquely associated with undifferentiated sarcomas [39]. Since then several other groups have published similar observations [42,43].

But here again one has to take this information with certain caution. We can argue that all that has been shown is that the gene expression profile of undifferentiated sarcomas corresponds closest to undifferentiated MSCs as compared to any known lineage committed sarcoma. However, it can be argued that this just means that the overall gene expression pattern is closest to MSCs but in fact it can be possible for these sarcomas to be committed to a lineage – but not progressed so far along that lineage to express a gene expression pattern that would identify them as such.

Delineating Differentiated from Undifferentiated: Commitment

The above models for the most part focus on degrees of differentiation. However, is there a point that separates undifferentiated from differentiating? In other words, theoretically speaking cells must begin to differentiate before one sees signs of the differentiation process. However, how can one judge a cell to be differentiating if not by some sign that is indeed doing so? This dilemma was what in the classical literature on differentiation has been referred to as “commitment.”

We have previously shown a role of Dkk1, a Wnt inhibitor, in the formation of undifferentiated sarcomas only. Although mouse models have helped elucidate the necessity for coordinated Wnt-signaling for bone, fat, cartilage, and muscle development, to the best of our knowledge it was Carl Gregory and Darwin Prockop [44] who first demonstrated the importance of Wnt-signaling to the undifferentiated MSC. More specifically, this group showed that the Wnt signaling inhibitor Dickkopf-1 (Dkk1) promotes MSC self-renewal and while maintaining their pluripotentiality. Thus, DDK1’s function as a maintainer of stemness and inhibitor of differentiation is highly compatible with its continued expression leading to the formation of undifferentiated sarcomas from MSCs. Similarly Wnt’s necessary function for proper skeletal development (e.g., Hill et al[45] selectively deleted β -catenin in limb and head mesenchyme, and showed that osteoblast precursors preferentially develop into chondrocytes; Glass et al[46] deleted β -catenin later in osteoblastic differentiation and found that the cells differentiate into osteoclasts) is completely compatible with its role in osteogenic sarcomagenesis (DKK1 is overexpressed) as recently demonstrated by Lee et al [47]. However, Wnt signaling has the opposite effect during adipogenesis (e.g., Longo et al[48] demonstrated that expression of a β -catenin agonist (Wnt10b) from the adipocyte specific *FABP4* promoter blocked development of both white and brown fat in mice).

We have additionally previously [39]** shown that epigenetic changes are important for sarcoma differentiation focusing on β -catenin – the transcriptional effector of Wnt signaling. Sequential chromatin immunoprecipitation (ChIP) experiments reveal that MSCs differentiated along the osteogenic lineage contain me-H3K4, the classical active chromatin mark, at the promoter regions of both c-myc and cyclin D1 (downstream Wnt/ β -catenin signaling targets) bound to β -catenin. Wnt/ β -catenin signaling promotes osteogenic differentiation [49], in agreement with this active me-H3K4 chromatin mark. Moreover, the repressive chromatin mark, me-H3K27, is missing from these osteogenic lineage-committed cells on c-myc and cyclin D1 promoters bound to β -catenin. Additionally, MFH cells treated with exogenous Wnt protein (to recapitulate Wnt signaling found in MSCs) and then differentiated along the osteogenic lineage reveal the presence of the active chromatin mark as well as the absence of the repressive chromatin mark on c-myc and cyclin D1 promoters bound to β -catenin. This is similar to MSCs differentiated along the same lineage, suggesting that MFH cells may originate directly from MSCs and so can be re-directed

along a terminal cell lineage to regain wildtype chromatin patterns. Conversely, Wnt/ β -catenin signaling is known to be inhibitory for adipocytic differentiation [50], and in MSCs differentiated along this lineage, the promoter region of cyclin D1 bound to β -catenin contains the repressive me-H3K27 mark and loses the active me-H3K4 mark.

Importantly, MSCs that are not lineage committed (before exposure to differentiation medium) contain both repressive and active chromatin marks on β -catenin-bound c-myc and cyclin D promoters. Similar results were obtained from MFH cells treated with exogenous Wnt protein (to recapitulate Wnt signaling found in MSCs). The simultaneous presence of both active and repressive histone marks is termed “bivalent chromatin” [51], and is thought to mark critical cell specification genes in embryonic stem cells. Bivalent chromatin modifications allow genes to remain in a repressed but quickly activatable state. This suggests that the MSCs and re-directed MFH cells maintain primed repression. Taken together, these results indicate that MFH cells can be modified not only to differentiate into mesenchymal lineages but also to recapitulate wildtype chromatin patterns on target genes.

Thus the presence of specific methylated histone patterns alone or in combination with other epigenetic modifiers may delineate the fine line between truly undifferentiated and differentiated states.

A little differentiated vs. undifferentiated: Does it matter?

For almost five decades the quest to define undifferentiated sarcomas has been an ongoing process. However, clinically speaking, soft tissue sarcomas whether undifferentiated pleomorphic or poorly differentiated are treated with the same set of cytotoxic chemotherapy [52]. Thus one has to wonder: does it make a difference whether a sarcoma is high grade undifferentiated pleomorphic or high grade undifferentiated lineage committed (e.g., dedifferentiated liposarcoma, high grade leiomyosarcoma, etc). Is this just an academic pursuit? We have reason and evidence to suggest the contrary.

Having shown that Dkk1 is overexpressed in undifferentiated sarcomas, and that it influences a canonical Wnt2 –signaling pathway required for commitment to mesenchymal differentiation; while Wnt5a/JNK non-canonical signaling regulates a commitment-viability-checkpoint [39,53]; we further examined whether expressing Wnt2 and Wnt5a in undifferentiated sarcomas cells was sufficient to recapitulate the overall pattern of Wnt-signaling observed in confluent MSCs. We postulated that this would allow for the controlled differentiation of undifferentiated sarcomas cells into mature connective tissue lineages. Undifferentiated sarcomas cells were cultured in the presence of both human recombinant Wnt2 (hrWnt2) and Wnt5a (hrWnt5a) for 72 hours prior to changing medium, and further growth in either adipocytic differentiation medium (ADM) or osteogenic differentiation medium (ODM) without hrWnt5a or hrWnt2. Only undifferentiated sarcomas cells pretreated with hrWnt2 and hrWnt5a readily accumulated markers of fat and mineralized calcium [39]. However a panel of osteosarcoma cell lines (lineage committed sarcomas) treated with just osteogenic differentiation medium showed a similar differentiation result (Matushansky et al, unpublished observations). This latter result suggests that truly undifferentiated sarcomas would require a commitment reprogramming step (in our model performed by manipulating the Wnt pathway); while lineage committed sarcomas would not require this additional step. This discrepancy would greatly change the therapeutic approach.

Mesenchymal Stem Cells and Sarcoma Stem Cells: Is there a link?

No review written today regarding the topic of “cell of origin” of any cancer, especially one that might arise from a tissue stem cell without evoking the issue of what is the relationship

between tissue stem cells and cancer stem cells and more broadly of course how does the cancer stem cell theory apply to a differentiation based model of solid carcinogenesis? The cancer stem cell theory states that there is a small subset of cancer cells, the cancer stem cells, which constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumor; and that these cancer stem cells have the capacity to both divide and expand the cancer stem cell pool differentiating into the heterogeneous nontumorigenic cancer cell types that in most cases appear to constitute the bulk of the cancer cells within the tumor [54].

In regards to sarcomas (as we have previously reviewed [53]), their “cancer stem cells” have not been thoroughly identified or characterized. While, several groups [55,56] have demonstrated spherocyte formation properties of some cell lines and have correlated spherocyte formation with expression of stem cell associated genes (e.g., Nanog, Oct4) and enhanced chemotherapy resistance, the definitive experiments that define stemness (i.e., serial dilution and sequential transplantation of a homogeneous sub-population followed by reestablishment of the heterogeneous tumor population [54]) have yet to be performed.

In writing of tissue stem cells and cancer stem cells, one should note of critical importance is that the cancer stem cell theory states nothing about the relationship between the tissue stem cell and the cancer stem cell. Furthermore as of this writing there is a lack of general consensus [57,58] as to whether or not cancer stem cells of differentiated tumors are simply tissue stem cells that retain genetic aberrancies that originated early in their development but not manifested until a later maturation point or whether cancer stem cells represent sub-clones of differentiating cells that have regained or reactivated an inherent stemness programming in a manner analogous to induced pluripotency [59,60]. Hopefully more work on this field will clarify the issue.

EXPERT COMMENTARY: Looking to the future: a speculative viewpoint on how the field may evolve

The sarcoma community has made much progress in elucidating the origins of MFH in the recent past if one considers that MHF was at best depicted by a state of the art review on sarcomas by Mackall et al [61] in 2002 with a question mark following it as arising out of a “stromal lineage”. While, we similarly believe that sarcomas are best thought of as tumors that arise from distinct stages of various differentiation lineages, we believe that recent data best supports the concept that MFH and undifferentiated sarcomas arise directly from the uncommitted MSC prior to the onset of differentiation. However, in our opinion to classify undifferentiated sarcomas as truly undifferentiated, as opposed to differentiated sarcomas with signs of commitment to a specific connective tissue lineage to evolve will require a break with the past methodologies of tumor categorization.

The classification of tumors lies within the domain of the pathologist. Pathology relies first and foremost on tumor cellular and architectural morphology. Immunohistochemistry and cytogenetics remain an adjunct to more narrowly define the diagnosis. However, to classify something that by definition has no obvious signs of differentiation (or at least not enough of them to be identified) will require to move away from morphology and immunohistochemical markers of the terminal phenotype, and to the identification of early markers of lineage differentiation. These would be markers that are expressed before the terminal phenotype could only be elucidated from understanding the molecular development of the corresponding lineage. However, even this is problematic for even if one can demonstrate that an undifferentiated tumor does not express any detectable levels of early markers for smooth muscle, fat, fibrocyte and a plethora of other lineages – how does one prove that it has no markers of all lineages?

Gene expression analyses has been proposed and demonstrated to be useful in classifying sarcomas (and other tumor types as discussed above). Advantage of this technique is that it does not require one to know any specific sets of genes or markers. A critical disadvantage is that it requires some arbitrary baseline. Consider the following problem. If one performs gene expression profiling on 10 MFHs, 10 dedifferentiated liposarcomas, 10 high grade leiomyosarcomas, etc; what one will observe is that not all of these tumor samples will associate as one would have predicted. In fact it has been our personal experience that the concordance rate between pathology classifications as performed by sarcoma-specializing pathologists both at Memorial Sloan Kettering and at Columbia is only about 80%. Furthermore, re-examination of tumor samples classifying outside of their initial classification by the pathologists have not been able shed any light on why these samples would misclassify via gene expression. Who is wrong? The pathologist or the gene expression analysis (the former would be wrong for subjective reasons while the latter of course would be wrong for technical reasons). MicroRNA based classification systems have recently started to gain stream and there have been some suggestions that they may be even better at classifying sarcomas than gene expression profiling [62].

In our opinion, the current histopathologic classification process is centered predominantly on morphology. Molecular methodologies (e.g., chromosomal breakpoint analysis for specific lymphomas and sarcomas) are seen as secondary determinants to assist with the primary modality of morphological classification. And while the current limited use of molecular methodology in pathology is indeed highly complimentary to morphologic classification, we are currently not taking enough advantage of the power of molecular methodology in its ability to truly synergize and not just compliment the morphologic classification. In order for the field to evolve we must be able to see beyond just morphology and to stop using morphology as the sole standard to which all else must concord. We have tried to do this by linking gene expression analysis of MFH and the various types of liposarcomas to an in vitro adipogenic differentiation time course. Although we set a non-subjective differentiation time line as our standard, to assert our hypothesis we nevertheless relied on groups of sarcomas that were designated as such, for the most part, solely by morphological criteria. In our opinion, future classification of sarcomas should rely on a system that gives equal weight to both morphological based determination and molecular methodology (i.e. gene expression/microRNA/epigenetic modification). Building on the potential synergism between morphologic classification and molecular methodology will we be able to truly differentiate the undifferentiated.

The other way in which we must break from the past is that we must be able to see through the heterogeneity of undifferentiated sarcomas, and to non-arbitrarily quantitate number of undifferentiated tumor cells versus differentiated tumor cells per tumor section. Our laboratory has previously reported on the use of 'systems pathology' focusing on computerized morphometric analysis to better define prostate cancer [63]. Although the mathematical algorithms that define morphometric analysis are extremely complicated, they in essence employ an image segmentation process in which objects (e.g., individual cells) are classified using spectral and shape characteristics. All cells are morphometrically analyzed for background (portion of the digital image that is not occupied by tissue), cytoplasm (amorphous pink area that surrounds an epithelial nucleus), nuclei (round objects surrounded by cytoplasm), spectral properties (color, brightness), and shape properties (length, width, compactness, and density); a mean for each parameter will be determined and normality defined as one standard deviation within the mean. Using this technique one can objectively quantitate the number and types of tumor and/or normal cells present within a give tumor.

In short, we envision that the field will evolve in such a way as to move away from morphology-alone based categorization and more towards integration of quantitative morphometrics and molecular parametrics (see Figure 1). This will undoubtedly prove a difficult transition, since it will require breaking from the past norms. However we believe that it must be done. Some might say that this is unrealistic and unreasonable. To quote Bernard Shaw, “The reasonable man adapts himself to the world; the unreasonable man persists in trying to adapt the world to himself. Therefore all progress depends on the unreasonable man.”

Key issues – 8–10 bullet points summarizing the review

1. In depth understanding of the biology of different mesenchymal tumor types is lacking, hindering our treatment options.
2. Murray and Stout characterize MFH and postulate that pleomorphic soft tissue tumors arise from histiocytes capable of fibroblastic transformation, the so-called “facultative fibroblast.”
3. MFH sub-divided into five subtypes: (1) storiform-pleomorphic, (2) myxoid (myxofibrosarcoma), (3) giant cell (malignant giant cell tumor of soft parts), (4) inflammatory, and (5) angiomatoid.
4. Concerns of MFH being a) a diagnosis of exclusion or b) a common morphological endpoint of many advanced tumors coupled with advances in diagnosis leads to re-classification of MFH into high grade undifferentiated pleomorphic sarcomas.
5. Gene expression profiling and functional analysis demonstrates that mesenchymal stem cells may be the precursors of MFH.
6. Epigenetic marking may be able to discriminate undifferentiated versus differentiated.
7. Differentiation therapy may be able to promote maturation of MFH into well differentiated tumors thus dramatically changing the prognosis of patients with the disease.
8. Further advances will depend on our ability to move beyond morphology-based pathology towards molecular pathology.

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Figure 1. Timeline of MFH highlighting critical trends past, present, and future. Please see text for details.