Research Article

Current Management and Molecular Targets of Synovial Sarcoma

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Abstract

Synovial Sarcoma (SS) is a rare and aggressive form of soft tissue sarcoma (STS) with a high metastatic potential that is characterized by a unique translocation between SYT on chromosome 18 and SSX on chromosome X. Presently, standard of care involves surgery, radiation therapy and chemotherapy. For those patients with metastatic disease, standard of care remains enrollment in a clinical trial. While there are numerous open clinical trials for the treatment of STS in general, clinical trials designed specifically for SS remain limited. The overall low response rate to cytotoxic chemotherapies has necessitated the need for development of pathway-specific targeted therapies for SS. Deregulation of several cell signaling pathways have been identified in SS, including the SRC, Bcl-2, and MDM2 signaling pathways, which are involved with cell growth, apoptosis, and p53 regulation, respectively. Additionally, several potential enzymatic targets have been identified, including argininosuccinate synthetase 1 and histone deacetylases. Here we present an updated review of the current therapy and the prospective molecular therapeutic targets that are available for clinical trial development in SS.

Keywords: Molecular Targets; Synovial Sarcoma; Deacetylases

Introduction

Synovial Sarcoma (SS) is a rare and aggressive form of soft tissue sarcoma (STS) with a high metastatic potential that frequently develops in young people between the ages of fifteen and forty [1,2]. The incidence of SS is estimated at 900-1000 cases per year in the United States and it accounts for 8-10% of the soft tissue sarcoma patient population. Though SS is not associated with an identifiable etiologic agent or genetic predisposition, it has been associated with a gene fusion product between transcription factors SYT and SSX1, SSX2, or SSX4. This translocation has been identified in 90-95% of all SS, and is pathognomonic and diagnostic for the disease [3]. Currently, the standard therapeutic approach to local primary disease and locally recurrent disease relies upon aggressive surgical resection, with neoadjuvant or adjuvant radiation and chemotherapy. However, in a majority of metastatic SS cases, clinical trial enrollment remains the standard of care with systemic chemotherapy remaining the sole therapeutic option off-trial. Unfortunately, the prognosis for patients presenting with metastatic disease remains poor, with a median time to cancer-specific death ranging from 10-22 months [1]. These findings highlight the need for more effective, less toxic systemic therapies for SS. In 2005, a well written work by Fukukawa et al. [4] analyzed several genes up-regulated in SS and postulated upon putative molecules for the development of novel therapies to treat SS [4]. Here, almost 10 years later, we present an updated review of the prospective molecular therapeutic targets for the treatment of SS.

Synovial Sarcoma Biology

Genetics /translocation biology

SS harbors a pathognomonic chromosomal translocation t(x;18) (p11.2;q11.2) that results in a fusion between the SYT gene on chromosome 18 and one of three homologous genes (SSX1, SSX2, SSX4) on the X chromosome (Figure 1). The SYT-SSX translocation
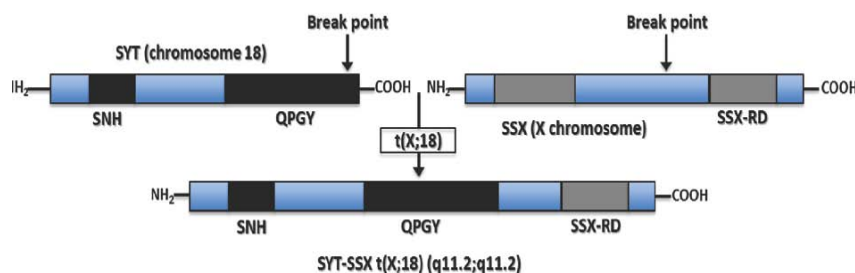
Figure 1: The SS translocation between the SYT transcriptional activator on chromosome 18 (Top Left) and the SSX transcriptional repressor located on the X chromosome (Top Right), specifically the translocation t(X;18) (p11.2;q11.2) (Bottom). The possible chromosomal rearrangements causing SS tumor genesis are SYT-SSX1, SYT-SSX2, and rarely SYT-SSX4.
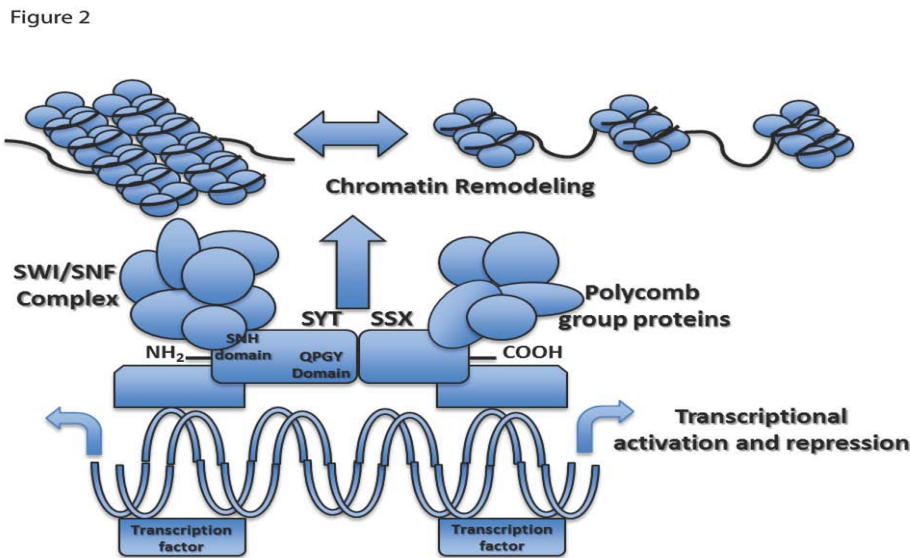
Figure 2: The SYT-SSX fusion protein alters gene expression level by modifying chromatin structure. The fusion protein associates with the SWI/SNF complex toaltersepigenetic regulation of a large subset of genes. Differential expression levels can be the result of deregulated histone methylation, acetylation, promoter methylation, or a number of other epigenetic mechanisms.

has been identified in approximately 95% of SS, and is the only cytogenetic abnormality in one-third of cases [3]. Most cases of SYT-SSX translocation associated SS harbor a fusion between SYT and SSX1 or SSX2, up to 10% of cases carry both translocations, and only rare cases carry the SYT-SSX4 translocation [5]. The anatomy of this SYT-SSX fusion oncogene has been extensively studied to better understand its pathogenicity, and it has been linked to aberrant E-cadherin repression, over expression of Bcl-2, and down-regulation of Mcl1 [6,7].

The SYT gene, also reported in the literature as SS18 or SSXT, is located on chromosome 18q11 and encodes a 387-amino acid protein. SYT is evolutionarily conserved and thought to function as a housekeeping gene given the presence of CpG islands and the absence of TATA elements in the promoter region [8]. The 54-amino acid N-terminal domain of SYT, referred to as the SNH domain, is believed to interact with SWI/SNF to alter chromatin remodeling and gene expression. SWI/SNF is a multi protein complex which counteracts repression by way of chromatin structural proteins such as histones and the polycomb-group of proteins (Figure 2) [8-11]. The C-terminal domain of SYT is rich in glutamine, proline, glycine, and tyrosine and is referred to as QPGY domain; it may function as a transcriptional activation domain on the basis of its similarity to corresponding domains in other transcriptional regulators [11].

The structure of SSX contains two major domains found in both SSX1 and SSX2; the N-terminal Kruppel-associated box (KRAB) domain, and the C-terminal dominant repressor domain SSX-RD [11-12]. The SSX-RD domain is the most highly conserved region of the protein among the various SSX1, SSX2, and SSX4 translocations. It is also believed to be required for the nuclear co-localization with the polycomb-complex that function to repress transcription through modification of higher-order chromatin structure [13].

The chimeric transcript in SS replaces the 5' portion of SSX with all but the eight C-terminal amino acids of SYT, but these eight amino acids do not appear to affect trans activation by the SYT QPGY domain. Together, the SYT-SSX fusion protein, in the SYT-QPGY and SSX-RD domains, displays both transcriptional activating and repressing domains, thus complicating hypotheses surrounding the oncogenicity of SYT-SSX [11]. Several studies have since demonstrated that the fusion protein is essential for tumor cell survival. Knockdown of the SYT-SSX1 protein decreases cell viability in SS; this finding of decreased viability is likely secondary to an increase in apoptosis [14-16]. Furthermore, re-introduction of a deleted exon 8 sequence into novel, patient-derived SS cell lines resulted in a decrease in cell viability as well, indicating that this splicing variant is important for the survival of SS [15].

Current Management

Although SS may develop at any anatomic site, they are found in the extremity in approximately 80% of cases, with lower extremities accounting for approximately 70% of cases [17]. In eight, seven, and five percent of cases, SS arise within the trunk, retroperitoneum/abdomen, and head/neck, respectively [18]. Cases in the extremities may predominantly involve the very distal aspect of either the hand/wrist or the foot/ankle and are often noted to be a palpable, slowly growing, and sometimes painful mass in the particular region of the joint. They rarely involve the actual joint, and, despite their name, are not associated with synovial tissue. Because of slow growth and insidious onset, there may be a delay in diagnosis, with one study citing an average of 2.5 years of symptomatology before patients sought medical care [19]. Once metastatic, SS are recognized to invade both locally at the primary site and distantly to the lungs and other sites. Intriguingly, SS has been shown to have a higher risk for lymph node metastases, with an incidence of 10-12% compared to approximately 3-5% for STS in general [20].

However, although there have been many published studies investigating the natural history of the disease, it has been difficult to draw clear conclusions in regards to the prognostic factors, treatment

outcomes, and survival statistics of SS in adults. This is most likely secondary to the rare incidence of the disease itself, the inclusion of children/adolescents in some retrospective studies, and variations in surgical and medical treatments. Although SS is viewed as moderately sensitive to chemotherapy, the 5-year distant recurrence rates, 5-year survival, and 10-year survival rates remain at 39%, 60% and 34%, respectively [1]. In 2000, Lewis et al published a retrospective analysis of 112 patients with localized primary extremity SS treated at Memorial Sloan-Kettering Cancer Center. These patients underwent surgical excision with curative intent, with only 22% requiring amputation. Chemotherapy and radiation therapy was administered only to 37% and 46% of patients, respectively, although treatment was not standardized. The 5-year recurrence rate was noted to be 39% in this cohort, with a majority of recurrences occurring in the lungs. In total, at 5 years, tumor related mortality was 25% for patients with primary extremity SS [21]. In regards to timing of recurrence, Singer et al in 1996 recognized a high rate of late metastasis, noting a 5-year survival rate of 60% and a 10-year survival rate of just 34%, with almost half of the disease-specific deaths occurring between 5 and 10 years [2]. Also, in those studies that have included patients with metastatic disease at the time of presentation, the median time to cancer-specific death ranges from 10-22 months [1,22].

Prognostic factors of disease-specific survival identified in multiple studies include age, size of tumor, margin status at resection, mitotic activity, bone or neurovascular invasion, histological subtype, p53 expression, Ki67 proliferative index, and SYT-SSX fusion type. Lesion size at presentation is variable, although most patients present with tumors larger than five centimeters. Those tumors that present at the periphery of a limb, for example, may be diagnosed earlier at a smaller size, although the rarity of the tumor and physical similarity to benign lesions may still result in a delay in diagnosis. In particular, large primary tumor size has consistently been associated with development of distant metastasis and decreased disease-specific survival [18]. Computed topography (CT) typically demonstrates a non infiltrative, well-circumscribed mass often with punctuate peripheral calcifications, although MRI is the modality of choice for diagnosis and initial staging of SS [23,24].

Pathological

Immunohistochemical and structural characteristics of SS cells are clearly different than those of synovial cells. SS have primarily been considered a high-grade tumor by definition, although some groups in Europe and Asia may classify SS as either high-grade or low-grade [25,26]. Morphologically, SS tumors can be composed of two distinct cell types in varying proportions. Each tumor typically consists of spindle cells and/or epithelioid cells, allowing for classification into three histologic subtypes: monophasic, biphasic, and poorly differentiated (Figure 3) [27,28]. The spindle cell component of a tumor consists of small, uniform, and ovoid cells with sparse cytoplasm and understated pale nuclei and nucleoli. The monophasic histologic subtype of SS tumors display primarily spindle cells with rare if any epitheiloid cells, arranged in intersecting fascicles with a hemangiopericytoma-like vascular pattern that often includes calcifications. Two-thirds of cases are of monophasic subtype [19].

In contrast, biphasic tumors consist of both spindle and epitheliod cell types, with the epithelioid component displaying cells with abundant cytoplasm and ovoid nuclei that often form glandular structures. The epithelioid component, when in glandular formation, displays features typical of adenocarcinoma; for example, lumina-containing epithelial mucin or papillary structures [27]. When monophasic tumors display the epithelioid subtype, cytogenetic analysis is usually required for diagnosis, as these tumors can be otherwise indistinguishable from adenocarcinoma [29]. Finally, the poorly differentiated histologic subtype of SS can be difficult to distinguish from other high-grade small-cell tumors, as they display rhabdoid features, dense cellularity, numerous mitotic figures, and areas of necrosis that canbe extensive [30].

Often, SS can be difficult to diagnose with routine histologic examination. Approximately 90% of tumors are cytokeratin-positive, with the epithelioid cell type staining stronger than the spindle cell type. Although SS are histologically similar to other spindle cell sarcomas, they stain positive for cytokeratin 7 and 19, which allows for reliable distinction between SS and primitive neuroectodermal and malignant peripheral nerve sheath tumors [31, 32]. Epithelial membrane antigens, which are expressed in SS but not in other spindle cell sarcomas, and Bcl-2, which is upregulated in SS, may also assist in immunohistochemical staining for diagnostic purposes [32]. Additionally, SS tumors stain positive for vimentin, supporting the hypothesis that SS cells are derived from multipotent stem cells of a mesenchymal and/or epithelial origin, rather than from synovial tissue as their moniker would suggest [33].

Molecular/Cytogenetic diagnosis

As SS can be difficult to diagnose through current clinical and histopathologic analysis, molecular testing should also be performed in cases with low to moderate clinical suspicion of SS [18]. Most biphasic tumors contain SS18-SSX1 while almost all tumors with SS18-SSX2 are monophasic. However, monophasic tumors have an equal chance of containing either SS18-SSX1 or SS18-SSX2
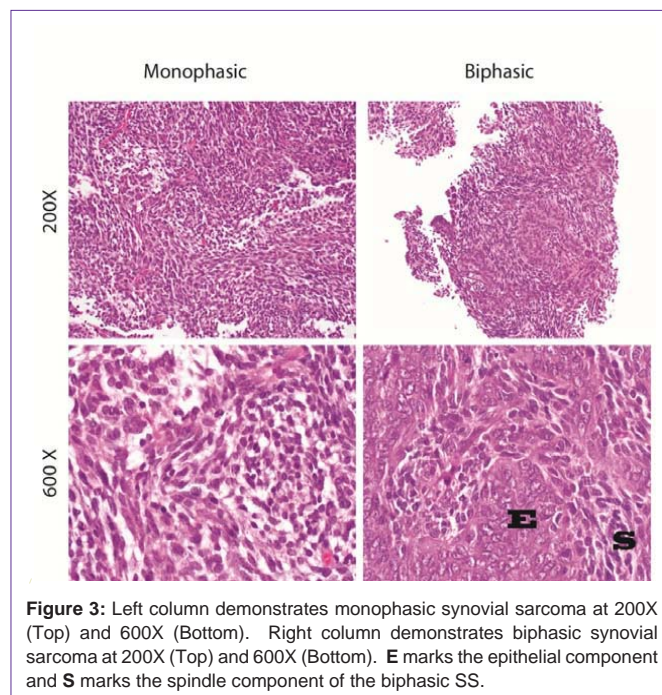
Figure 3: Left column demonstrates monophasic synovial sarcoma at 200X (Top) and 600X (Bottom). Right column demonstrates biphasic synovial sarcoma at 200X (Top) and 600X (Bottom). **E** marks the epithelial component and **S** marks the spindle component of the biphasic SS.

[34]. A tumor with a SS18-SSX translocation may be diagnosed via fluorescence in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR) [29]. These diagnostic modalities may be performed on frozen or paraffin-embedded clinical samples [35,36,37].

Current Therapy

Surgery

The foundation for treatment of STS, as well as SS, is complete surgical resection with wide margins [18]. Standard of treatment dictates that non-curative surgery, such as an intra-lesion resection or incomplete gross resection should not be offered or attempted. As SS are most commonly diagnosed in an extremity and in proximity to a joint, careful dissection of tissue planes and neurovascular structures is necessary. Fortunately, it is uncommon that amputation of an extremity is required as primary surgical therapy [19]. Of those patients who undergo surgery for locally recurrent disease, limb-sparing surgery is possible for the majority of patients, although greater morbidity involving critical structures like nerves, veins, arteries, or even bone may be expected [19]. Even in cases involving a limb, amputation is still considered a last resort [38].

Surgical therapy for patients with metastatic disease requires careful patient selection to ensure benefit; considerations include patient performance status, extent of disease burden, disease-free interval, and response to systemic chemotherapy. For those patients with pulmonary metastasis, complete resection has been shown to improve survival [39].

Radiation therapy

Radiation therapy has been employed for SS in the neoadjuvant and the adjuvant setting. Preoperative radiation therapy in the form of external beam radiation is used to reduce tumor size at resection, but is associated with reduced dose, smaller fields, and a higher risk of long-term wound complications [19]. Radiation is typically used in the adjuvant setting for patients with tumors over five centimeters or for areas where local tumor control may be difficult, as in the head and neck. At this time, the use of concurrent systemic chemotherapy and radiation is investigational [19].

In patients who experience local recurrence, the re-irradiation of a site is typically not feasible, as those patients have received maximum radiation dosage to that site. However, the use of brachytherapy may be possible with minimal morbidity in patients with prior radiation. A group at Memorial Sloan-Kettering Cancer Center has reported the largest experience with the use of brachytherapy and interstitial after loading brachytherapy. In a study investigating the local control of surgery and brachytherapy compared to surgery alone, local control was 95% versus 54%, significantly higher in those who received brachy therapy [40]. In a study from 1990, Nori reported an overall 5-year actuarial local control of 68% with the use of interstitial brachytherapy; however, this study included patients with various types of recurrent sarcomas who experienced varying numbers of recurrences [41].

Chemotherapy

SS are considered to be moderately chemotherapy sensitive, and are among the more chemotherapy sensitive STS. Anthracycline-based chemotherapy employing doxorubicin was the first chemotherapy agent to display activity against SS, although response rates of doxorubicin combinations approach 45% at 10 years, there is no statistically significant difference in overall survival noted compared to sequential therapy [42].

For those patients who present with metastatic disease, clinical trial enrollment is considered the standard of care. For the treatment of metastatic disease off trial, ifosfamide, anthracyclines and pazopanib are the most active agents. Some physicians choose to increase the dose of ifosfamide in the setting of recurrence if the patient has been previously treated with an ifosfamide regimen [19].

Pazopanib is an oral angiogenesis inhibitor that targets vascular endothelial growth factor receptors (VEGFRs), platelet-derived growth factor receptors (PDGFRs), and c-kit. In a phase II study from the European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC Study 62043) in which 37 SS patients were enrolled [43], progression free rate at twelve weeks of treatment was reported as the primary end point, and 49% of patients with SS demonstrated stable disease. In 2012, a multi-center phase III study also designed by EORTC, 369 patients with angiogenesis inhibitor-naive, metastatic STS, progressing despite previous standard chemotherapy, were randomly assigned to receive pazopanib or placebo. Median progression-free survival for SS patients was longer for pazopanib compared with placebo (4.1 months versys 0.9 months) [44].

Current Clinical Trials

As no targeted therapy for SS has yet to be developed, enrollment in clinical trials is recommended as standard of care at this time. For patients newly diagnosed with all subtypes of STS, there are many clinical trials enrolling at this time. The number of open clinical trials specific to SS, however, remains limited. A phase I study of genetically engineered NY-ESO-1 specific (c259) T cells in HLA-A2+ patients with SS opened, with the purpose of testing the effects of chemotherapy and the NYESO T cells on patients with metastatic and recurrent SS [45].

The potential of NY-ESO-1 as a cancer therapeutic target relies on the concept of specific immune recognition of cancer, and the subsequent development of an anti-cancer response. The NY-ESO-1 gene is found on the X chromosome at q28, and it codes for several products, namely NY-ESO-1, a 180-amino acid protein. Expression pattern analysis by RT-PCR for NY-ESO-1 has confirmed that the protein in normal tissue is restricted to testis, but found frequently in cancer. The function of the protein is yet to be determined; however, the presence of NY-ESO-1 is noted in one-third to one-fourth of all melanoma, lung, esophageal, liver, gastric, prostate, ovarian, or bladder cancers [46]. Strikingly, approximately 80% of SS have been found to express NY-ESO-1 [47], which holds promise for immunotherapeutic approaches such as NY-ESO-1 specific T cells as noted above. In this phase I study, the primary outcome measure is to determine whether the administration of T cells genetically engineered to recognize a peptide derived from NY-ESO-1 in HLA-A2+patients demonstrate a response rate consistent with that observed using similar NYESO-1 specific T cells plus aldesleukin in patients with SS. This trial is open to patients who are HLA-A2+ with SS that have been treated with standard chemotherapy with
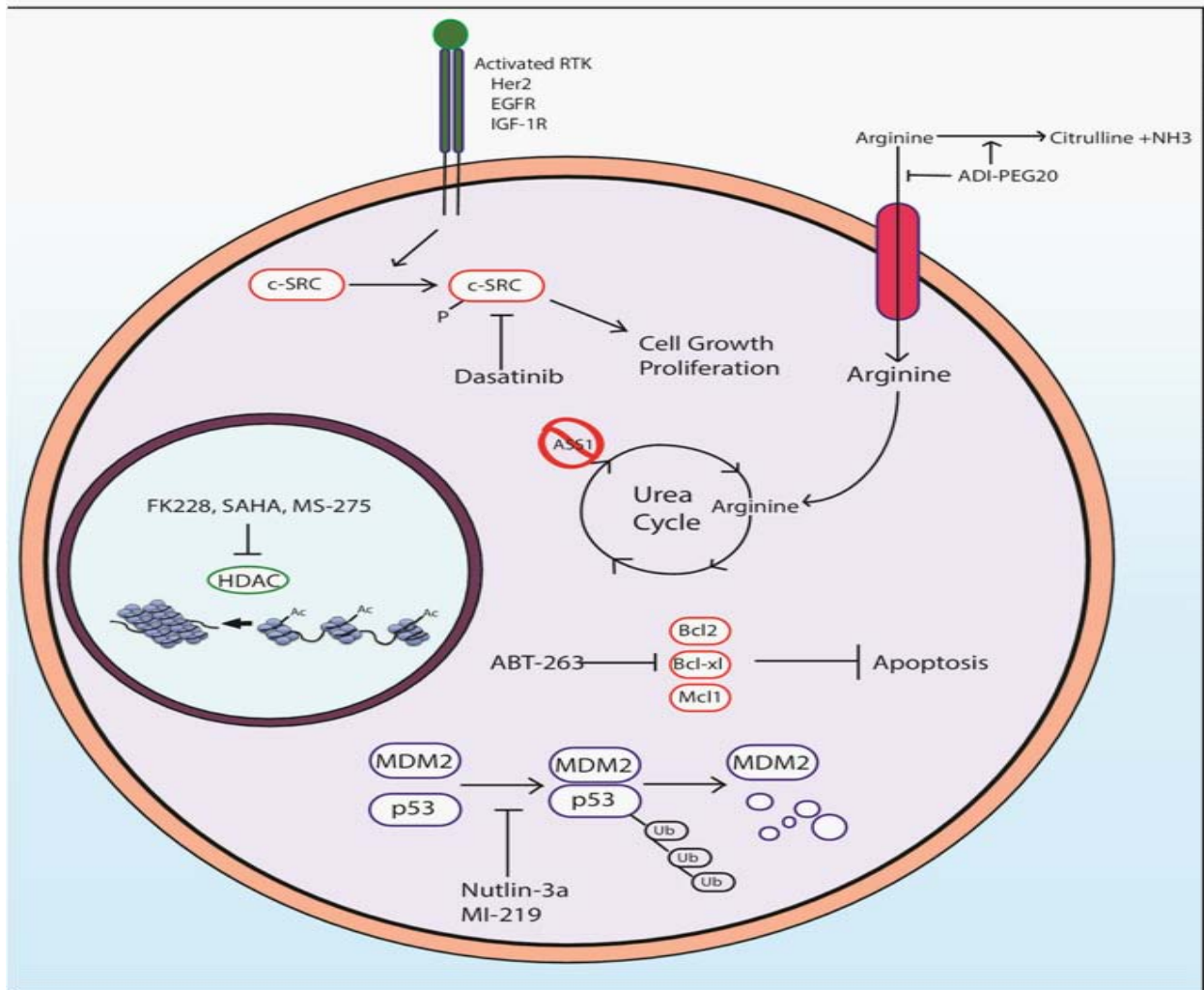
Figure 4: A schematic of the possible therapeutic targets for SS. Reliance upon SRC signaling suggests inhibition of upstream receptor tyrosine kinases as well as SRC itself can provide therapeutic benefits. The epigenetic silencing of ASS1 can be targeted via depletion of extracellular arginine. As SS utilizes Bcl-2 over expression in order to increase resistance to apoptosis, targeting with Bcl-2 inhibitors will induce apoptosis in tumor cells. MDM2 amplification causes p53 poly-ubiquitination and proteasomal degradation, thus inhibition of the MDM2-p53 interaction can increase downstream p53 signaling. Lastly, inhibition of HDACs has been shown to increase expression of tumor suppressor genes silenced via histone deacetylation.

remaining measurable disease that is metastatic, progressive/persistent, recurrent, or unresectable. In September of 2013, the NCI opened a second clinical trial for enrollment involving NY-ESO-1 in order to determine whether the administration of anti-ESO mTCR-engineered peripheral blood lymphocytes plus high-dose aldesleukin following a non-myeloablative lymphoid depleting regimen will result in objective tumor regression in patients with metastatic cancers that express the ESO antigen, including SS [45].

Onco Therapy Science Inc, opened a clinical trial in France for patients with histologically confirmed progressive SS that is resistant to doxorubicin and ifosfamide in order to investigate a chimeric humanized monoclonal antibody against FZD10, named OTSA101. The gene encoding frizzled homologue 10 (FZD10), a 7-transmenbrane receptor and member of the Wnt signalling receptor family, is overexpressed in SS and is undetectable in normal human tissues except placenta. Non-radio labeled OTSA101 antibody has only weak antagonistic activity on SS cell growth *in vivo*. However, Yttrium 90-radiolabeled OTSA101 (OTSA101-DTPA-90Y) showed significant antitumor activity following a single intravenous injection in mouse xenograft model. This study should allow for definition of the optimal recommended dose of this novel monoclonal antibody therapy [48].

Molecular Targets for Clinical Trials

SRC: c-SRC (SRC) is a non-receptor tyrosine kinase involved in regulation of cell growth, survival, and motility [49]. The kinase has two phosphorylation sites, with phosphorylation of Tyr527 reducing kinase activity, and autophosphorylation of Tyr416 inducing full kinase activity [50,51]. SRC phosphorylation at Tyr527 is regulated by c-SRC tyrosine kinase (CSK) and the protein tyrosine phosphatase PTP1B [52,53]. Deregulation of CSK, as well as overexpression

of PTP1B, have been shown to be critical in a number of cancers, including colon cancer and breast cancer [54-56]. SRC has also been shown to interact with a number of receptor tyrosine kinases, including insulin like growth factor-1 receptor (IGF-1R), and effectors of the PI3K/AKT, RAS/MAPK, and STAT3 pathways. SRC interaction with focal adhesion kinase (FAK), various integrins, and regulators of the Rho-GTPases allows regulation of cellular migration [57-61].

Deregulation of SRC signaling is crucial for growth and survival of SS cells. SRC Tyr416 phosphorylation has been shown to be one of the strongest phosphorylated kinases in SS cell lines [49]. The high level of Tyr416 phosphorylation is induced by the SS translocation SYT-SSX, which has been shown to up-regulate IGF-1R, EGFR, and Her2 signaling [58,62-64].

The SRC inhibitor dasatinib has been shown to inhibit growth in SS cell lines, as well as increase apoptosis and decrease the mitotic rate in SS cells. Combination of dasatinib with conventional chemotherapy drugs has shown additive effects in SS cell lines [49]. Inhibitors of IGF-1R, EGFR, or Her2 signaling may also be suitable targeted therapies for SS due to their ability to decrease SRC signaling (Figure 4).

Bcl-2: The Bcl-2 protein family is important in regulation of apoptosis, and frequently found to be mutated in a number of cancers. Bcl-2 is one of the antiapoptotic members of the family, along with Mcl-1, Bcl-xl, and a number of other proteins [65,66]. The family includes apoptosis promoting proteins, including Bad, Bax, and Bid, among others. By alternatively regulating the permeability of the mitochondrial membrane, the proapoptotic Bcl-2 proteins can cause cytochrome c release from the mitochondria and subsequent initiation of apoptosis. The BH3 domain is the 'death domain', found in all proapoptotic Bcl-2 proteins and capable of introducing a pore into the mitochondrial membrane to allow cytochrome c release.

SS has deregulated expression of a number of proteins of the Bcl-2 family, including both antiapoptotic and proapoptotic proteins [6]. Part of the SS expression signature includes high level of Bcl-2 expression, leading to apoptotic resistance [67-69]. Bcl-xl, another antiapoptotic protein, has also been shown to be up regulated by the SYT-SSX fusion protein. Mcl-1 and Bcl2a1a, both antiapoptotic proteins, are down regulated by SYT-SSX expression [6]. Up regulation of anti-apoptotic proteins likely contributes to the resistance of SS to many cytotoxic chemotherapy.

Targeted therapeutics directed towards the apoptotic pathway have been developed capitalizing on the BH3 death domain of the Bcl-2 proapoptotic proteins. BH3 domain peptidomimetics have been shown to be capable of targeting SS cell lines and xenografts and inducing apoptosis. ABT-263 is a potent Bcl-2 inhibitor and capable of sensitizing SS to classical cytotoxic chemotherapies [6] (Figure 4). The lack of Mcl-1 expression, the natural therapeutic bypass to the Bcl-2 inhibitors, makes Bcl-2 a highly attractive target for the treatment of SS.

MDM2: MDM2, or mouse double minute 2 homolog, is a key protein in the p53 signaling pathway. MDM2 functions as an E3 ubiquitin ligase [70]. Under normal cellular conditions, MDM2 binds to and poly-ubiquitinates p53, marking the transcription factor for

proteasomal degradation. When the cell experiences a p53 activating signal, including DNA damage, oxidative stress, or ribonucleotide depletion, MDM2 function can be inhibited by a number of mechanisms to up regulate p53 function and subsequently up regulate p53 target genes [71]. MDM2 can be inhibited by phosphorylation or localization to the nucleolus by p14arf, the protein product of the alternative reading frame of the p16 INK4a locus. Activation of p53 can cause transcription of genes causing cell cycle arrest or apoptosis depending on the strength and duration of the activating signal [72].

While SS are typically wild type for p53, an up regulation of MDM2 has been documented [73]. The SYT-SSX fusion protein is capable of increasing p53 poly-ubiquitination and subsequent proteasomal degradation by increasing the stability of MDM2. MDM2 auto-ubiquitination is inhibited in SS, leading to increased MDM2 stability [74]. The increase of MDM2, and subsequent decrease of p53, leads to an increase in the ability of SS cells to survive typical apoptotic signals by attenuating the response of the p53 activating signal. The decreased level of p53 leads to the increased resistance of apoptosis in response to genotoxic stress in SS.

Since SS is typically wild type for p53, therapeutics targeting the activity of MDM2 can be particularly useful in treatment of SS due to their ability to reactivate the p53 signaling axis [75]. Small molecule inhibitors of MDM2 in combination with cytotoxic chemotherapy may provide enhanced toxicity to SS [72,76]. Two such small molecule inhibitors of this interaction include nutlin-3a and MI-219, both of which have been shown to decrease MDM2-p53 interaction, increasing the transcriptional activation potential of p53 [70] (Figure 4).

ASS1: ASS1, or argininosuccinate synthetase 1, is a key enzyme in the urea cycle, catalyzing the formation of argininosuccinate from citrulline and aspartate. Cells that are deficient in ASS1 expression rely upon arginine from the environment. When deprived of arginine, these cells are incapable of producing the amino acid and undergo autophagy [77,78]. It has been shown that in nearly 90% of sarcomas, the ASS1 promoter is heavily methylated and expression is subsequently silenced [79]. ASS1 methylation has been shown to correlate with reduced metastasis free survival. These tumors have been shown to be arginine auxotrophs, with inhibited growth and induction of autophagy upon arginine deprivation [80,81].

SS have been shown to be one of the classes of sarcoma in which ASS1 is silenced [9]. The reliance upon extracellular arginine can be exploited with use of ADI-PEG20. This pegylated form of arginine deiminase is capable of converting extracellular arginine into citrulline and ammonia and has been shown to decrease arginine levels in the blood stream and cause ASS1 deficient tumors to undergo autophagy. ADI-PEG20 has also been shown to increase the radio sensitivity of ASS1 deficient tumors, and thus may increase the susceptibility of SS to traditional radio therapy [82] (Figure 4).

HDAC: Histone deacetylases, or HDACs, are important chromatin remodeling enzymes involved in the epigenetic regulation of the genome. Histone tail acetylation decreases the electrostatic interaction between the histone tail and the DNA backbone, allowing the two to dissociate, enabling transcription. Thus, acetylation of histones correlates with increased gene expression, while deacetylation correlates to tighter associations between DNA and

histones, condensation of the chromatin, and subsequently decreased gene expression. Epigenetic down regulation of tumor suppressors have been shown to contribute to SS cell growth and proliferation.

As SYT-SSX is a chromatin remodeling fusion protein, targeting this epigenetic deregulation has been shown to inhibit SS cell growth *in vitro* and *in vivo* [83]. The HDAC inhibitors have also been shown to induce apoptosis in SS cell lines. HDAC inhibitors are capable of driving the re-expression of genes typically silenced in tumors, including the p21/WAF1 cyclin dependent kinase inhibitor [84-86]. The HDAC inhibitors that are currently in clinical trials for cancer treatment, including FK228, MS-275, SAHA, and PXD101 could prove to be very effective in the targeted treatment of SS [87-89] (Figure 4).

Summary

The standard of care for the treatment of sarcoma is clinical trial when surgical resection is not possible. The unique biology of SS driven by its hybrid transcription factor SYT-SSX makes it ideal for histology driven clinical trials. Optimal candidates for therapy include the HDACs, SRC, MDM2, ASS1, and Bcl-2 (Figure 4, Table 4). The most likely successful strategy will involve targeting more than one of these unique SS inhibited targets simultaneously. Combinations such as MDM2 inhibition with an HDAC inhibitor or a Bcl-2 inhibitor with ADI-PEG20 and the various combinations that are possible wait direct testing in SS cell lines and patients. Rationally designed therapies based on the specific biology of SS should lead to a bright future for the treatment of SS.

Acknowledgements

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