Review *Clin Ter 2023; 174 Suppl. 2 (6):68-76 doi: 10.7417/CT.2023.2473*

Omics sciences and precision medicine in sarcoma

G. Bonetti[1,2], K. Donato[3,4], K. Dhuli[1], M. Gadler[1], S. Benedetti[1], G. Guerri[1], F. Cristofoli[3], D. Generali[5], C. A. Donofrio[6,7], M. Cominetti[6], A. Fioravanti[6], L. Riccio[6], A. Bernini[8], E. Fulcheri[9], D. Cavalca[10], L. Stuppia[11,12], V. Gatta[11,12], S. Cristoni[13], S. Cecchin[1], G. Marceddu[3], M. Bertelli[1,3,4]

[1]MAGI'S LAB, Rovereto (TN), Italy; [2]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy; [3]MAGI EUREGIO, Bolzano, Italy; [4]MAGISNAT, Peachtree Corners (GA), USA; [5]Department of Medicine, Surgery and Health Sciences, University of Trieste, Italy; Multidisciplinary Unit of Breast Pathology and Translational Research, Cremona Hospital, Italy; [6]Department of Neurosurgery, ASST Cremona, Cremona, Italy; [7]Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [8]Department of Biotechnology, Chemistry, and Pharmacy, University of Siena, Siena, Italy; [9]Fetal-Perinatal Pathology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy; Department of Surgical Sciences and Integrated Diagnostics, Università di Genova, Genoa, Italy; [10]Laser Surgery Operating Unit, Plastic Surgery Department, San Rocco Clinical Institute, Ome, BS, Italy; [11]Department of Psychological Health and Territorial Sciences, School of Medicine and Health Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy; [12]Unit of Molecular Genetics, Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy; [13]ISB Ion Source & Biotechnologies srl, Bresso (MI), Italy

Abstract

Background. Sarcomas are a relatively rare but diverse group of cancers that typically develop in the mesenchymal cells of bones and soft tissues. Occurring in more than 70 subtypes, sarcomas have broad histological presentations, posing significant challenges of prognosis and treatment. Modern multi-omics studies, which include genomics, proteomics, metabolomics, and micro-biomics, are vital to understand the underlying mechanisms of sarcoma development and progression, identify molecular biomarkers for early detection, develop personalized treatment plans, and discover drug resistance mechanisms in sarcomas to upsurge the survival rate.

Aim. This study aims to highlight the genetic risk factors responsible for sarcoma-genesis, and to present a comprehensive review of multi-omics studies about sarcoma.

Methods. Extensive literature research was undertaken using reliable and authentic medical journals, e-books, and online cancer research databases. Mendelian inheritance in man database (OMIM) was explored to study particular genes and their loci that are responsible to cause various sarcomas.

Result. This in-depth research led to the finding out that omics studies provide a more comprehensive understanding of underlying molecular mechanisms of sarcomas. Through genomics, we can reveal genetic alterations that predispose to sarcoma, like mutation in TP53, NF1, and so on. Pharmacogenomics enable us to find molecular targets for specific drugs. Whereas, proteomic and metabolomic studies provide insights into the biological pathways involved in sarcoma development and progression.

Conclusion. Future advancements in omics sciences for sarcoma are on the cutting-edge of defining precision treatment plans and improved resilience of sarcoma patients. ***Clin Ter 2023; 174 Suppl. 2 (6):68-76 doi: 10.7417/CT.2023.2473***

Key word: *sarcoma, omics sciences, genomics, genetics, metabolomics, microbiomics, proteomics, cancer*

Introduction

Sarcoma is a histologically diverse group of malignant tumors that develop in connective tissues of fat, bones, cartilage, and muscles (1). Predominantly, sarcomas originate in mesenchymal stromal cells (MSC) of the bone marrow, which are undifferentiated stem cells, causing osteoblasts, adipoblasts, chondroblasts, and several other connective tissue cells (2).

Sarcomas are referred to as 'rare' tumors because of their low incidence rates. Until recently, annual occurrence of sarcoma is <6/100,000 individuals worldwide (1,3,4). The majority of sarcoma sufferers are young children (accounting for >20% of total pediatric), whereas only 1% of all older adults malignancies are connected to sarcoma (1,4).

To date, there have been about 100 different types of sarcomas described in the 2020 WHO classification of tumors, each of which is etiologically and pathologically distinct in said publication (3,5). This new classification has upgraded clinical decision-making, correct pathological diagnosis, surveillance and management of this heterogenous cancer (5). For the sake of convenience, scientists segregated sarcomas in three broader classes, namely: bone sarcoma, soft tissue sarcoma (STS, which occurs in muscles, nerves, retroperitoneum, blood vessels, originating mostly in extremities), and visceral sarcoma (occurring in specific organs, like the gastrointestinal tract) (4,6). Among these multiple varieties, the majority of diagnosed sarcoma originate in soft connective tissues, mostly in extremities (60% of cases), with over 50 histological subtypes, whereas 10% of sarcoma subtypes belong to bone sarcoma (4,5).

Correspondence: Gabriele Bonetti. MAGI'S LAB, Rovereto (TN), Italy. Email. gabriele.bonetti@assomagi.org. Tel. +39 036562061

ISSN 1972-6007

Although the exact etiologies and causes of many STSs are not well-understood, certain risk factors have been recorded to increase the risk of sarcomas. Environmental risk factors like exposure to ionizing radiation, carcinogenic chemicals (like arsenic, anabolic steroids, and thorium), virus infection, prior tumor experience, increased body mass index, age, diabetes, and obesity are associated with a higher risk of developing sarcoma (4,6,7). Genetic anomalies responsible to cause STSs include the inactivation of tumor suppressor gene because of germline mutations, like in neurofibromatosis and nerve sheath tumors. Sarcoma genesis may occur due to hereditary genetic predisposition of genes, like in Li-Fraumeni-syndrome (6,7).

The discovery of diagnostic biomarkers and treatment targets for cancers is indispensable, but identifying the basic tumor driving forces, like genomic alterations, and their impact on sarcoma phenotypes remain major challenges. "Omic" sciences are helping in this regard, as they provide high-throughput approaches to assess metastasis phenotypes and chemotherapy resistance and to find therapeutic targets. Genomics, metabolomics, transcriptomics, and proteomics research can lead to a better insight into the oncogenesis, improved prognosis, and personalized tumor management (8).

Clinical presentation and diagnosis of sarcoma

Identical to the diverse nature of sarcoma, the clinical presentation of each STS patient is highly variable. Sometimes a lump or a mass appears on bones and extremities, and every so often sarcoma may remain unnoticed. Rare typical symptoms of STSs include fever, weight loss, and weakness (9).

Because of STSs' rarity and histologic overlaps, diagnosing them is a great deal. Primary diagnosis is done using medical imaging (CT scan, MRI) combined with biopsy investigations from the affected tissue (9)(4). In addition to conventional topography and morphology-based strategies, molecular pathogenetic testing has revolutionized STS diagnosis. Staging of STS is done on the basis of 'grades'. This grading system has been suggested by the French Federation of Cancer Centers Sarcoma Group (FNCLCC), which is also approved by WHO (4,10). Grades are best to describe necrosis and mitotic activity in cancers, and serve as powerful prognostic tools to predict metastasis in STS (4,10).

Molecular genetic analyses—like fluorescence in situ hybridization, reverse transcription polymerase chain reaction, and sequencing of targeted genes—are advancements in routine diagnosis of STS (11). Moreover, genomic screening and high-throughput targeted sequencing have broadened the landscape of precision oncology of sarcomas and its therapeutic goals (12). Although histopathological findings serve as the backbone of STS diagnosis, the potential utility of recent molecular profiling and omics information through immunohistochemical markers also improves pathological diagnosis of STS (11). These methods make it easier to find new therapeutic targets to formulate personalized medical care for STS (12,13).

Genetic susceptibility of sarcomas: cancer predisposition syndromes

Identifying genetic causes of sarcomas is still difficult, because most STS types—such as liposarcoma, synovial sarcoma, angiosarcoma, and Ewing sarcoma—occur sporadically (14,15). However, Mendelian Randomization (MR) approaches to elucidate underlying causal associations of STS with familial gene segregations have been documented in many studies and case reports. MR is the analysis of germline genetic variants, like single nucleotide polymorphisms (SNPS), and has an edge over other conventional observational studies. It helps to specifically identify risk factor of interest by measuring the genetic variability, independent of other biological pathways, that is randomly assigned at conception (16).

Somatic cell mutations happen after meiosis and are confined to cancerous cells, while germline mutations occur in all the cells of an organism. When cancers are observed in families with consistent pattern (Mendelian genetic pattern), they are regarded as having familial cancer predisposition syndrome. Various sarcomas are attributed to arise from heritable cancer predisposition syndromes (14,15).

Familial cancer predisposition syndromes significantly contribute to premature mortality (17). Those who survive after STSs are at an increased risk of developing any other form of cancer. So, it is advisable to turn the testing of germline variants that predispose to sarcoma into a routine clinical practice for entire suspected families and individual members diagnosed with STS. This will help in grasping the disease's natural condition and the patients' therapeutic needs, thus tailoring their personalized treatments. Also, it will enable the family members to seek genetic counselling and figure out their cancer risk (15).

A study highlighted two germline pathways that are mainly responsible to cause mesenchymal cancers (18). One involves the variation in centrosome genes during a mitotic division; the other is the heritable variations in shelterin complex (six telomer-associated proteins). This study confirms the findings of prior studies, showing that pathogenic variants occurring in mitosis and telomeres are heritable and enriched in STS patients (17,18).

There has been a longstanding association between sarcoma and cancer predisposition syndromes. Most of the pathogenic germline variants of CPGs are observed in TP53, NF1 and BRCA1/2 genes (15,17). Here, we are briefly discussing some selected heritable cancer predisposition syndromes that elicit sarcomas. An overview of the genes causing various sarcomas has been summarized in Table 1 below (14)(19):

1. **POT1 tumor predisposition (POT1-TPD)**
   It develops when a heterozygous pathogenic variant POT1is identified in a proband by molecular genetic testing. It increases the risk of developing multiple cutaneous melanomas, gliomas, chronic lymphocytic leukemia, and, particularly, angiosarcoma. First-degree relatives of suspected patients should also be tested for POT1-TPD, as it is genetically transferred as an autosomal dominant disorder. Each offspring of such patients would have 50%-increased chances of getting POT1-TPD phenotypic spectrum (20).

Table 1. Selected inherited genetic syndromes with genes that cause sarcomas (14)(19)

Inherited Syndromes	MIMs of syndrome phenotypes	Genes	Cytogenetic location	Gene OMIMs	Emerging Sarcoma	Inheritance
APC, Gardner syndrome (Familial adenomatous polyposis)	175100	APC	5q22.2	611731	Desmoid tumors	AD
Beckwith-Wiedemann syndrome	130650	ICR1, KCNQ1OT1, CDKN1C	11p15.5, 11p15.5, 11p15.4	616186, 604115, 600856	Embryonal rhabdomyosarcoma (RMS)	AD/ sporadic
Bloom	210900	RECQL3	15q26.1	604610	Osteosarcoma, Embryonal RMS	AR
Carney-Stratakis	606864	SDHB, SDHC, SDHD	1p36.13, 1q23.3, 11q23.1	185470, 602413, 602690	GIST	AD
Constitutional mismatch repair syndrome	619101	PMS2	7p22.1	600259	Embryonal RMS	AR
Costello	218040	HRAS	11p15.5	190020	Embryonal RMS	AD
Familial GIST	606764	SDHB, SDHC, KIT	1p36.13, 1q23.3, 4q12	185470, 602413, 164920	GIST	AD
Familial pleuropulmonary blastoma (DICER1 syndrome)	601200	DICER1	14q32.13	606241	Embryonal RMS	AD
Familial rhabdoid predisposition syndrome	609322	SMARCB1	22q11.23	601607	Malignant rhabdoid tumor	AD/ somatic
Gorlin syndrome/nevoid basal cell carcinoma syndrome	109400	PTCH2, PTCH1, SUFU	1p34.1, 9q22.32, 10q24.32	603673, 601309, 607035	Embryonal RMS	AD
Hereditary retinoblastoma	180200	RB1	13q14.2	614041	Osteosarcoma, STS	AD
HLRCC	150800	FH	1q43	136850	Uterine leiomyosarcoma	AD
Li Fraumeni Syndrome	151623	TP53	17p13.1	191170	Osteosarcoma, RMS, STS	AD
Mosaic variegated aneuploidy	257300	BUB1B	15q15.1	602860	Embryonal RMS	AR
Multiple osteochondromas	133700	EXT1	8q24.11	608177	Chondrosarcomas	AD
Neurofibromatosis 1	162200	NF1	17q11.2	613113	MPNST, GIST, RMS	AD
Nijmegen breakage syndrome	251260	NBN	8q21.3	602667	Embryonal RMS	AR
Noonan syndrome	163950	PTPN11	12q24.13	176876	Embryonal RMS, giant cell tumor of bone, granular cell tumor, PVNS	AD
Rothmund-Thomson syndrome- II	268400	RECQL4	8q24.3	603780	Osteosarcoma	AR
Rubinstein-Taybi	180849	CREBBP	16p13.3	600140	Embryonal RMS, LMS	AD
Tuberous sclerosis	191100	TSC1	9q34.13	605284	PEComa tumor (Pacoima), chordomas	AD
Werner syndrome	277700	RECQL2	8p12	604611	Osteosarcoma, embryonal RMS	AR

Abbreviations: *AD, autosomal dominant; APC, adenomatous polyposis coli; AR, autosomal recessive; GIST, gastrointestinal stromal tumor; HLRCC, hereditary leiomyomatosis and renal cancer; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; NF1, neurofibromatosis type1; PEComa, perivascular epitheloid cell tumor; RCC, renal cell carcinoma; RMS, rhabdomyosarcoma; STS,*

2. NTHL1 tumor syndrome

It develops in patients who are diagnosed with germline biallelic pathogenic variant in NTHL1. Diagnosis is made using molecular genetic testing, and it runs genetically as autosomal recessive manner. It increases the risk of getting colorectal cancer, breast cancer, and colorectal polyposis. It is advisable to check relatives, even those who are asymptomatic, for early diagnosis and appropriate treatment (21).

3. **Rhabdoid tumor predisposition syndrome**
   Malignant rhabdoid tumors develop when mutation occurs in SMARCB-1 or SMARCA4 genes. It increases the risk of rhabdoid tumors, which are malignancies mainly of the nervous system ad brain, but they can also occur at any anatomical location. It predominantly develops in infants before three years of age. It is genetically transferred in autosomal dominant manner. Pathogenic germline variant SMARCB1 has de novo disease causing ability and can be diagnosed without a family history of such tumors (22).
4. **DICER1 tumor predisposition**
   Mutation in germline DICER1 pathogenic variant leads to an increased risk of developing pleuropulmonary blastoma, thyroid gland neoplasia, thyroid cancer, ovarian tumors, and cystic nephroma. Occasionally, it may lead to cause rhabdomyosarcoma and central nervous system sarcoma. It is inherited in autosomal dominant fashion, and relatives should be screened to overcome future pathogenic circumstances. Early detection and genetic counselling are keys to surveillance (23).
5. **Li-Fraumeni syndrome (LFS)**
   This is an inherited condition or cancer predisposition syndrome that increases the chances of getting various types of childhood- and adult-onset tumors in an individual. Five commonly observed cancers in Li-Fraumeni syndrome are osteosarcoma, soft tissue sarcoma, adrenocortical carcinomas, breast cancer, and central nervous system cancers. The risk of developing cancer with LFS is greater in females (>90%) than in males (>70%).
   The variant that is responsible to cause LFS is the occurrence of a mutation in TP53 gene, and it is inherited in autosomal dominant fashion in an individual whose family members have experienced an array of various cancers in a single ancestral line (24,25). As TP53 pathogenic variant loses its tumor suppression, leading to malignancy and toxicity, there is also evidence suggesting that genomes that are enriched in functional copies of TP53 may halt the development of cancer (14).
6. **Lynch syndrome**
   This cancer predisposition syndrome increases the risk of developing colorectal cancer and cancers of biologically vital organs, like stomach, ovary, bowel endometrium, urinary tract, skin, brain, pancreas, biliary tract, and sarcomas. Lynch syndrome is the result of a mutation in genes MLH1, MSH2, MSH6, PMS2, and EPCAM. It is inherited in autosomal dominant fashion. Lynch syndrome encompasses a spectrum of different sarcoma types, such as fibrous histiocytomas, rhabdomyosarcomas, liposarcoma, and leiomyosarcomas (25,26).
7. **Hereditary diffuse gastric cancer**
   It is an autosomal dominant disorder, caused by alteration in CDH1 pathogenic variant. It increases the risk of developing diffuse gastric cancer. It is an adenocarcinoma, characterized by infiltration and thickening of the stomach wall without any grown mass or lesion. Another rare gastric tumor may be predisposed as a result of pathogenic variation in PRKAR1A in Carney complex or Carney syndrome. This gastrointestinal stromal tumor was previously termed as "gastric epitheliod leiomyosarcoma" (25).
8. **Neurofibromatosis type 1 or NF1**
   NF1 disorder is one of the most commonly occurring human predisposition syndromes, with a frequency of 1 in 3,000 individuals. Prominent features of NF1 are the growth of numerous nerve sheath tumors (called neurofibromas) and marks of cutaneous hyperpigmentation at various sites (called café-au-lait spots). Sometimes axillary freckling, bone dysplasia, optic glioma, and iris hamartomas may also be clinical findings. Pathogenic variant NF1gene—responsible for producing the tumor suppressor neurofibromin—loses its functionality, leading to spontaneous mitogenic signaling via mitogen-activated protein kinase pathways (14,27).
   NF1 disorder increases the susceptibility to develop a highly aggressive soft-tissue sarcoma called malignant peripheral nerve sheath tumor (MPNST), which accounts for 2% of all STS. NF1 syndrome may also give rise to another mesenchymal tumor, the gastrointestinal stromal tumors (GISTs), in the interstitial cells of Cajal present at the lining of GIT. Children affected by NF1are also at greater risk of developing rhabdomyosarcoma (14,27).
9. **Familial adenomatous polyposis (FAP)**
   Principally, it is a colorectal cancer syndrome that can be identified with numerous adenomas, also called polyps, throughout the lining of the large bowel. These polyps have great potential to transform into a sarcoma called fibromatosis (28). FAP is inherited as autosomal dominant syndrome, resulting from germline alteration in adenomatous polyposis coli (APC) gene. APC is a tumor suppressor that is responsible to regulate the levels of β-catenin, which controls mitogenic signaling. FAP runs in families with 100% penetrance, and patients are likely to develop colorectal cancer after the age of 40. FAP patients predominantly predispose to other complex cancers called desmoid tumors (14).
   Desmoid tumors are fibroblastic neoplasms that usually occur in abdominal walls, mostly in the peritoneum. They are locally aggressive, but rarely metastasize. Their severity is variable, from painless lesions to greatly invasive tumors that can lead to mortality. FAP patients are 15 % more likely to develop desmoid tumors (14,29).
   The correspondence between distinct sarcomas with different heritable cancer predisposition syndromes is naturally complex. Exploring these associations is the center of interest for the formulation of personalized oncologic therapeutics.

Genomics of sarcoma: unraveling of Pharmacogenomics (PGx) biomarkers

Due to the complex genetic origin and histological intricacies of sarcoma, its prognosis is generally poor, and devising effective drugs for its treatment poses significant challenges. Primarily, localized STS is treated by surgically removing the cancer mass, with subsequent radiotherapy, while tumor progression and metastasis are controlled via chemotherapeutics. Nevertheless, significant results to encounter diverse forms of STSs were not achieved, and the survival rate after metastatic STS is as low as 30% after two years of treatment. The reason is the difference

in pharmacological response to drugs, which is unique in every patient (30).

Molecular mechanisms that cause sarcoma initiation

In literature, there are three fundamental molecular mechanisms that have been observed to cause sarcoma-genesis. First of all, malfunctions of gene expression that occur due to anomalous, chimeric transcription factors, resulting from characteristic gene fusions in translocation-associated sarcomas. Secondly, mutations that occur in vital signaling pathways can also propel sarcoma. Lastly, aberrations in DNA copy-number (31).

Novel research on sarcoma is uncovering important genetic information and identifying specific point mutations occurring along with translocations, oncogenes that are lineage-specific, events that remodel chromosomes, and genetic alterations that affect normal signaling and differentiation pathways (31).

Pharmacogenomic (PGx) biomarkers discovery and their clinical application

As each patient's genetic make-up is unique, it will produce a different idiosyncratic response to multifactorial drugs, which can be tricky to foretell. Polymorphic variants in genes responsible for drug absorption, distribution, metabolism, and excretions (ADME) influence the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug. These PKs and PDs intervene at different biological levels, including metabolome, epigenome, transcriptome, and proteome, giving distinct clinical outcomes. Such unpredictable drug responses, due to polymorphic variants in genes, directly affect drug dosage, efficacy, toxicity, chances of hypersensitivity, and drug resistance (32).

Pharmacogenomics (PGx), sometimes called pharmacogenetics, have been utilized in genetics with the ultimate goal of identifying 'genomic variations' that can offer valuable insights for enhancing drug effectiveness and minimizing the risks of chemotherapy-related side effects (33). Numerous studies and clinical trials have shown interindividual unique responses to certain drugs.

Recently, the correlation between common genetic alterations and drug responses have been explored and published widely for large cohorts of individuals from diverse populations, and are included in the Genome Wide Association (GWAS) catalog, generated by the National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI) (32). Genomic profiling has led to developing specialized pharmacogenomic biomarkers that help to foresee specified drug responses and figure out genetic basis, possible risks of toxicity, and differences in treatment efficacy for STS patients (30,34).

The germline alterations in the genome of patients, especially single nucleotide polymorphisms (SNPs), are highly penetrant predisposed mutations that serve as the potential biomarkers for drug-induced toxicity and drug response. Also, cancer predisposing somatic mutations that occur randomly due to DNA damage have been impeccably utilized as drug targets (30,34). These pharmacogenomic (PGx) biomarkers, which can predict efficacy and any possible adverse drug reactions, are incorporated in membrane transporters, drug targets, drug-metabolizing enzymes, and HLA alleles (30,34).

Here, we are selectively discussing some valuable pharmacogenomic biomarkers, that have been extensively implicated and studied for STS patients.

– The human solute carrier family (SLC) are membrane transport proteins responsible to carry inorganic ions, lipids, neurotransmitters, amino acids, and drugs. From this family of membrane transporters, organic cations transporter (OCTs) and nucleoside transporters (NTs) have been widely studied for STS, suggesting that OCT6-mutation may have long lasting effects on PKs and PDs of doxorubicin in breast cancer patients (34,35).

– Human equilibrative nucleoside transporter or h-ENT1 also belong to SLCs, and is found in erythrocytes, brain, placenta, mammary glands, and other soft tissues. It is a nucleoside transporter that influences the absorption of pyrimidine-based drugs like gemcitabine (34). A retrospective study, performed on leiomyosarcoma and angiosarcoma patients, was carried out to understand the link between hENT1 expression and clinical response of gemcitabine: it showed that high levels of hENT1 are linked with better clinical results of gemcitabine in sarcoma patients (34).

– ATP-binding cassette (ABC) superfamily consists of seven different classes of membrane proteins that are involved in multi-drug resistance; thus, they can cause cancer treatment failure by efflux of antineoplastic molecules. In this family, ABCB, ABCC, and ABCG subtypes are widely studied for sarcomas. ABCB encodes p-glycoprotein (Pg-p), whose expression levels are found remarkably high in many tumor cells—including STS, breast, gastric, kidney, leukemic, and liver cancers. Conventional drugs used for the treatment of STS include anthracyclines, taxanes, and tyrosine kinase inhibitors like imatinib and sorafenib. Their responses are significantly affected by high levels of Pg-p (34,36).

– ABCC family consists of six pumps, with abundance of MRP1, which maintains cellular resistance to anthracyclines; MRP2 reduces oral absorption and enhances hepatobiliary clearance of drugs. A study performed on 60-year-old male STS patients showed that SNPs were prominent in ABCC family and adverse events with trabectedin (34,37).

– Pyrimidine metabolism is a key component of DNA/RNA, important for phospholipids and protein metabolism, and also serves as a target of many chemotherapeutic regimens. Its antagonists—like 5-fluorouracil, gemcitabine, and cytarabine—have been shown noticeable success by inhibiting its synthetic enzymes, like nucleoside monophosphate kinase (UMPK/CMPK). CMPK plays an important role in the synthesis of cytidine analogs and is the potential target of gemcitabine-based chemotherapies for leukemia, solid tumors, and lymphoma. (34).

– Cytochrome P450 family, a group of oxidative enzymes that are claimed to metabolize anti-cancer drugs, exhibiting remarkable variations in its genes (CYP). A study was conducted to observe the effects of cyclophosphamide in rhabdosarcoma patients: results showed that carrying mutant CYP2B6 alleles affected the patient's response to cyclophosphamide. Patients who carried only one mutation

(in particular SNP of CYP2B6) showed better impression of the drug, while patients with three alleles of mutant SNPs exhibited short event-free survival (34).

– Breast cancer 1 gene (BRCA1) encodes for tumor suppressor protein. It works in response to DNA damage, and mutations in this gene indicate hereditary breast and ovarian cancers. It also serves as an important biomarker for sarcoma, which has been noted to give improved prognosis and disease management for sarcoma patients. Increased expression of BRCA1 is linked with lower response to trabectedin (34,38).

– Role of sodium Dichloroacetate (SDA) as an anti-tumor agent

Anti-oxidant enzymes and pro-oxidant processes perform key functions in the development of sarcoma. A study measured the activities of anti-oxidant enzymes in sarcoma-affected tissue homogenates of mice that are treated with SDA. Results showed that SDA minimized the activities of oxidative enzymes playing key roles in tumorigenesis, thus demonstrating anti-cancer effects of SDA (39).

A well-known property of cancer, including sarcomas, is the presentation of Warburg effect, in which the glycolytic pathway is extraordinarily activated even in the presence of oxygen, leading to enhanced tumor growth (40). This aerobic glycolysis is an inefficient way for ATP synthesis to fulfill the demands of uncontrolled proliferating cells, thus creating an imbalance of nutrients and energy for somatic cells. To overcome or reverse this Warburg effect, dichloroacetate (DCA) is widely utilized to treat sarcoma, which is a pyruvate dehydrogenase kinase (PDK) inhibitor, since it potentially restricts tumor growth and reduces the resulting apoptosis. Research and clinical trials have verified the efficacy of this treatment in managing sarcoma (41).

Immuno-pharmacogenomics

Immunomodulatory pathways in sarcomas can be potential targets for immunotherapy, in combination with tailored therapy. Next generation sequencing methods are utilized for genetic profiling of host immune cells in the emerging field of immunogenomics or immune-pharmacogenomics, also revealing their potential to enhance immunotherapy efficacy, as well as to serve as a mediator for the activity of cytotoxic agents and targeted drugs. This new approach holds promise for providing valuable information to predict clinical outcomes and to monitor the treatment response of sarcoma. Moreover, it may help identify tumor neoantigens that could be targeted for novel immunotherapies (42).

For instance, Poly (ADP-ribose) polymerase-1 inhibitors (PARP1-i) are responsible to activate immunomodulatory pathways in STS cells and also to alter tumor microenvironment. For this reason, PARP1-i is an attractive candidate to combine with immune checkpoint inhibitors (ICI), which will ultimately improve efficacy and provide effective therapy for tumors. Likewise, T-cells of upgraded affinity have also been transferred in the patients (especially in synovial sarcoma) for tumor-specific antigens and have given promising results (34).

For planning customized treatments for the patients and to sidestep unwanted toxicities of drugs, it is highly necessary to identify and develop absolute pharmacogenomic biomarkers with the goal to maximize therapeutic benefits, as the genuine success of getting personalized medicine for sarcoma patients is associated with the discovery of pharmacogenomic biomarkers that will help to stratify patients as responsive and non-responsive subjects. It will also make it possible to find out whether the patients have chances to develop treatment-associated toxicity (33).

Metabolomics of sarcoma

Metabolomics of biomolecules facilitate the study of metabolite concentrations and their widespread outcomes in metabolic pathways, reflecting the associations between the genotype and the phenotypes of a cell. The implication of metabolomics in sarcoma has enabled the researchers to identify the involvement of specific metabolic pathways and their alterations in the development and progression of these tumors. Such studies of different types of sarcomas revealed distinct metabolic signatures associated with each subtype (8). Furthermore, applications of this tactic also provided insights into the mechanisms of drug resistance in sarcomas, as well as helped identify potential therapeutic targets (43).

Cell cycle deregulation in sarcomas occurs due to deviations from major metabolic pathways, like glycolysis, biosynthesis of macromolecules (like amino acids and nucleotides), and mitochondrial respiration. Major oncogenic alterations include boosted glycolysis, glutaminolysis, and oxidative phosphorylation in sarcomas, increasing ATP production and establishing chemoresistance. As a whole, STS display overexpression of tyrosine kinase receptor (Her4) and activation of RAS, PI3K, and HIPPO pathways, coupled with a predominant glycolytic/oxidative phosphorylation signature. The cancer genome atlas (TCGA) database reveals unique metabolic profiles in individual STS subtypes as compared to other cancers. For instance, enhanced PPAR/fatty acid and glycine/serine/threonine pathways are features of UPS subtypes, whereas increased OXPHOS levels are observed in LMS subtypes (44).

Glucose metabolism in sarcoma

Glucose homeostasis is crucial to be maintained by two pathways, named glycolysis and gluconeogenesis (45). Fructose bis-phosphatase (FBP) is an important rate-limiting enzyme of these pathways, converting fructose 1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate. It is extensively found in muscles and mesenchymal cells. The role of fructose-1,6 bis-phosphatase (FBP) is reported in sarcoma—its loss being a key event in sarcoma development—and it is an active target for anti-sarcoma drugs (46).

Numerous studies corroborated that cancer cells, including STS, exhibit the ubiquitous feature of metabolizing glucose through Warburg effect in times of biosynthetic requirements of nutrients. In tumors, glucose uptake is remarkably enhanced by aerobic glycolysis, in which, instead

of making pyruvate as an end product and transporting it to mitochondria, cancer cells switch glycolysis cycle to make lactate, which is released in extracellular matrix (45). This leads to an acidic microenvironment that facilitates tumor growth and invasiveness, also decreasing mitochondrial activity, which can cause oxidative stress and DNA damage. The key regulating enzyme of Warburg effect, lactate dehydrogenase-A (LDH-A), may be a promising target of therapies in certain types of STS that exhibit overexpression of LDHA (44,45).

Amino acid metabolism

Various amino acids (like glutamine, arginine, and tryptophan) have been studied in the metabolomics of STS. Predominantly, glutamine and arginine metabolic pathways are found to promote STS progression (44). Glutamine is metabolized by mitochondrial glutaminase (GLS), a rate-limiting enzyme of glutaminolytic pathway. Its activity is examined in different STS subtypes, including undifferentiated pleomorphic sarcoma (UPS), synovial sarcoma, and leiomyosarcoma, which showed elevated protein expression of GLS in these tumors (45). Therefore, GLS inhibitors have also been experimented in vivo and in vitro, demonstrating the upsurge of cell cycle arrest, reduction in cell proliferation, and augmentation in cell death, thus showing dependency of tumor cells on glutamine metabolism (46). Similarly, arginine metabolism in sarcoma showed that sarcoma cells lack the rate-limiting enzyme of this semi-essential amino acid, which is required for its synthesis. Tumor cells thus become arginine deficient, leading to starvation and metabolic stress (46).

Presently, many studies have reported the potential of integrated genomic and metabolomic stratagems for interpretation of tumor complexity. A recent article from The Cancer Genome Atlas (TCGA) research network proposed a new classification STS subtypes by combining genetic, epigenetic, and transcriptomic analyses. They evaluated copy number variations (CNVs) and identified three dominant profiles from leiomyosarcoma (LMS), myxofibrosarcoma (MFS), and undifferentiated pleomorphic sarcoma (UPS) that exhibited the maximum number of genomic alterations. Moreover, epigenetic apparatus, by activating pathways and immune signatures, complements prognostic value (44).

Several studies evidenced that STS growth affects mitochondrial fitness. As it depends upon the availability of key metabolites, glycolysis, glutaminolysis and oxidative phosphorylation, disruption of various mitochondrial pathways occurs. For example, in major STSs, a disturbance of TCA cycle occurs, due to the alterations in the expression of its enzymes, such as SDH and FH, and to the excess of oncometabolites. Eventually, it leads to tumor chemoresistance, poor prognosis, and heterogeneity in mitochondrial activity (44).

Proteomics of STS

Research suggests that a proteome holds more than 1000-folds cellular information than the genome, which is transcribed to more than 100,000 transcripts and gives rise to millions of protein variants or protein isoforms because of alternative splicing (AS), single amino acid polymorphisms (SAPs), and wide-ranging post-translational modifications (PTMs). Thus, proteome is a promising field to study the cellular systems in carcinomas, having the potential to reveal valuable biomarkers and drug targets (47).

Proteomics involve the whole protein fractions of a cell and address post-translational modifications (PTMs) and protein-protein associations. Next-generation drug discovery approaches are aimed to target protein inactivators, such as proteolysis targeting chimeras (PROTACs) and immune-oncology agents. Proteomic tools consists of mass spectrometry and protein array methodologies to measure myriads of PTMs in proteome and diverse physiochemical properties of amino acids (48).

In a study, tyrosine phosphorylation (pY) signaling pathways were observed in sarcoma subtypes. 10 different histologically distinct subtypes are studied including osteosarcoma, rhabdomyosarcoma, leiomyosarcoma, and Ewing's sarcoma. Authors of this study identified some exceptional phosphorylation proteins, platelet derived growth factor receptor alpha (PDGFR), and some other unique signaling proteins, whose expression was found elevated in distinct sarcoma subtypes. Some other studies also experimented the identification of phosphoprotein biomarkers, which are helping in patient stratification and prognosis (46).

Microbiomics of sarcoma

These days, microbiome interactions with immune system have gained much importance in defining antitumor immune responses. The reason is that microbiome (genes of microorganisms, along with cellular environment entities) plays a crucial role in the progression of tumors, and gut microbiome configuration affects the clinical outcomes of immune checkpoint-blockade therapy. A growing body of research suggests that an intratumoral microbiome exists, since it was observed that in many cancers it contributes to cancer advancement and interferes with immunotherapy responses (49).

A recent study by Perry et al. (2023) detected the viral microbiome of an intratumoral soft tissue sarcoma, for the first time in a prospective STS cohort utilizing a firmly sterile collection protocol. They found a unique and noticeable microbiome, namely a *Respirovirus*, in tumor samples of STS patients, that exhibited reproducibility and correlation with natural killer cells infiltration. They observed a complex interaction between tumor microenvironment (TME), host immune system, and intratumoral microbiome. In general, natural killer (NK) cells, the activators of the innate immune system, are able to target both cancerous and virally infected cells upon stimulation. Herein, viral microbiome is linked with higher NK cells infiltration inside the tumor, which may provide a useful link for prognosis. This may also contribute to the provision of potential therapeutic target in immunotherapy resistant STS patients. The viral microbiome was found in high levels in patients with local STS, and low levels in patients with metastasis (49).

In another study, distinct fungal population or mycobio-

me has also been detected in 35 cancer types, mostly found in cancer cells and immune cells. Those fungal ecologies exhibited specificity with age, tumor subtypes, smoking, response to immunotherapy, and survival rate. The authors also detected multiple mycobiome-bacteriome-immunome complexes and determined their co-occurrence in tumors. Categorically, they acknowledged fungi-stimulated pan-cancer mycotypes, with characteristic immune responses that will help to stratify patient survival and clinical outcomes. Firm positive interactions observed between fungal and bacterial varieties, obtained from several cancer types, suggest the existence of multi-domain microbial colonization in tumor microenvironment (50).

Previously, similar findings about the presence of bacterial population have also been reported a few decades ago, in a patient of Kaposi's sarcoma (51). Another study by Xin et al (2022) backed up the hypothesis that micro-organisms favor carcinogenesis. Xin and colleagues found abundant tumor-associated fungal populations in diverse cancer types, which strengthened the notion that fungi are ubiquitous to all major cancers; moreover, their specific type can be predictive of endurance (52).

In the next few decades, multi-genomic studies on sarcoma would be primarily focused on the development of precision medicines, as they rely on identifying candidate genes and their mutations, which are susceptible tumor drivers involved in phenotypic presentations, drug resistance, and metabolic alterations in tumors. Tailored therapy of STS aims to collect omics data about the patient as a whole, and molecular info of developed cancer, and match them with a medicine with highest compatibility and minimal side effects (33). Research on omics biomarkers will lead to early monitoring of recurrence of STS and will also be helpful for finding novel therapeutic targets.

Conclusion

The implication of Omics sciences in sarcoma research holds great promises for improving our understanding of the underlying causes of these tumors, grasping the disease's natural condition and the therapeutic needs of STS patients, thus tailoring a personalized treatment for each of them. Specifically, the links between cancer predisposition genes (CPGs) and sarcomas may be a landmark to early diagnosis, screening, and genetic counselling, thus leading to anticipation strategies for index patient and their family. Not only, studying such links can restrain the increasing risk of malignancies, and can also help in developing personalized medicines for STS patients.

Acknowledgements

This research was funded by the Provincia Autonoma di Bolzano in the framework of LP 14/2006.

Conflicts of interest statement

Authors declare no conflict of interest.

References

1. Mackall CL, Meltzer PS, Helman LJ. Focus on sarcomas. Cancer Cell. 2002 Sep;2(3):175-8
2. Morozov A, Downey RJ, Healey J, et al. Benign mesenchymal stromal cells in human sarcomas. Clin Cancer Res. 2010 Dec 1;16(23):5630-40
3. David BBL, Abdon Mello C, Santos Thuler LC, et al. Overview of Adult Sarcoma Burden and Clinical Pathways in Brazil. JCO Glob Oncol. 2022 Mar;8:e2100387
4. Alorjani MS, Matalka II, Alfaqih MA, et al. Soft Tissue Sarcomas: A 16-Year Experience of a Tertiary Referral Hospital in North Jordan. Medicina (Kaunas). 2022 Jan 27; 58(2):198
5. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO Classification of Soft Tissue Tumours: news and perspectives. Pathologica. 2021 Apr;113(2):70-84
6. Weskamp P, Ufton D, Drysch M, et al. Risk Factors for Occurrence and Relapse of Soft Tissue Sarcoma. Cancers (Basel). 2022 Mar 1;14(5):1273
7. Thomas DM, Ballinger ML. Etiologic, environmental and inherited risk factors in sarcomas. J Surg Oncol. 2015 Apr;111(5):490-5
8. Min L, Choy E, Tu C, et al. Application of metabolomics in sarcoma: From biomarkers to therapeutic targets. Crit Rev Oncol Hematol. 2017 Aug 1;116:1–10
9. Ilaslan H, Schils J, Nageotte W, et al. Clinical presentation and imaging of bone and soft-tissue sarcomas. Cleve Clin J Med. 2010 Mar;77 Suppl 1:S2-7
10. Coindre JM. Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med. 2006 Oct;130(10):1448-53
11. Schaefer IM, Cote GM, Hornick JL. Contemporary Sarcoma Diagnosis, Genetics, and Genomics. J Clin Oncol. 2018 Jan 10;36(2):101-110
12. Jacobs C, Lapeire L. Translating Molecular Profiling of Soft Tissue Sarcomas into Daily Clinical Practice. Diagnostics (Basel). 2021 Mar 14;11(3):512
13. Gounder MM, Agaram NP, Trabucco SE, et al. Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma. Nat Commun. 2022 Jun 15;13(1):3406
14. Farid M, Ngeow J. Sarcomas Associated With Genetic Cancer Predisposition Syndromes: A Review. Oncologist. 2016 Aug;21(8):1002-13
15. Würtemberger J, Ripperger T, Vokuhl C, et al. Genetic susceptibility in children, adolescents, and young adults diagnosed with soft-tissue sarcomas. Eur J Med Genet. 2023 May;66(5):104718
16. Lustbader ED, Williams WR, Bondy ML, et al. Segregation analysis of cancer in families of childhood soft-tissue-sarcoma patients. Am J Hum Genet. 1992 Aug;51(2):344-56
17. Mandelker D, Ladanyi M. Germline study points to sarcoma pathways. Science. 2023 Jan 20; 379(6629):238-239
18. Ballinger ML, Pattnaik S, Mundra PA, et al. Heritable defects in telomere and mitotic function selectively predispose to sarcomas. Science. 2023 Jan 20;379(6629):253-260
19. Gene Map Statistics - OMIM (Accessed August 4 2023, at https://www.omim.org/statistics/geneMap)
20. POT1 Tumor Predisposition - PubMed (Accessed August 4 2023, at https://pubmed.ncbi.nlm.nih.gov/33119245/)
21. Kuiper RP, Nielsen M, Voer RM De, et al. NTHL1 Tumor Syndrome. GeneReviews® (Accessed August 4 2023, at https://www.ncbi.nlm.nih.gov/books/NBK555473/)
22. Nemes K, Bens S, Bourdeaut F, et al. Rhabdoid Tumor Predisposition Syndrome. GeneReviews® (Accessed

August 4 2023, at https://www.ncbi.nlm.nih.gov/books/NBK469816/)
23. Schultz KAP, Stewart DR, Kamihara J, et al. DICER1 Tumor Predisposition. GeneReviews® (Accessed August 4, 2023, at https://www.ncbi.nlm.nih.gov/books/NBK196157/)
24. Schneider K, Zelley K, Nichols KE, et al. editors. GeneReviews(®) (Accessed August 4 2023, at https://www.ncbi.nlm.nih.gov/books/NBK1311/)
25. Poultsides G, Norton JA. Hereditary Diffuse Gastric Cancer. Gastric Cancer Princ Pract (Accessed August 4 2023, at https://www.ncbi.nlm.nih.gov/books/NBK1139/)
26. Kohlmann W, Gruber SB. Lynch Syndrome. GeneReviews(®) (Accessed August 4 2023, at https://www.ncbi.nlm.nih.gov/books/NBK1211/)
27. Ferrari A, Bisogno G, Macaluso A, et al. Soft-tissue sarcomas in children and adolescents with neurofibromatosis type 1. Cancer. 2007 Apr 1;109(7):1406-12.
28. Clark SK, Johnson Smith TGP, Katz DE, et al. Identification and progression of a desmoid precursor lesion in patients with familial adenomatous polyposis. Br J Surg. 1998; 85(7):970–3
29. Calvert GT, Monument MJ, Burt RW, et al. Extra-abdominal desmoid tumors associated with familial adenomatous polyposis. Sarcoma. 2012; 2012
30. Caruso C, Garofalo C. Pharmacogenomics Biomarkers of Soft Tissue Sarcoma Therapies. Front Oncol. 2020 Apr 15;10:509.
31. Taylor BS, Barretina J, Maki RG, et al. Advances in sarcoma genomics and new therapeutic targets. Nat Rev Cancer. 2011 Jul 14;11(8):541-57
32. Arbitrio M, Scionti F, Di Martino MT, et al. Pharmacogenomics Biomarker Discovery and Validation for Translation in Clinical Practice. Clin Transl Sci. 2021 Jan;14(1):113-119
33. Serra M, Hattinger CM. The pharmacogenomics of osteosarcoma. Pharmacogenomics J. 2017 Jan;17(1):11-20
34. Gambale E, Boddi A, Pasqui A, et al. Pharmacogenomics of soft tissue sarcomas: New horizons to understand efficacy and toxicity. Cancer Treat Res Commun. 2022 Jan 1;31:100528
35. Lal S, Wong ZW, Jada SR, et al. Novel SLC22A16 polymorphisms and influence on doxorubicin pharmacokinetics in Asian breast cancer patients. Pharmacogenomics. 2007 Jun;8(6):567-75
36. Molina-Ortiz D, Torres-Zárate C, Cárdenas-Cardós R, et al. MDR1 not CYP3A4 gene expression is the predominant mechanism of innate drug resistance in pediatric soft tissue sarcoma patients. Cancer Biomarkers. 2018 Jan 1;22(2):317–24
37. Laurenty AP, Thomas F, Chatelut E, et al. Irreversible hepatotoxicity after administration of trabectedin to a pleiomorphic sarcoma patient with a rare ABCC2 polymorphism: a case report. Pharmacogenomics. 2013 Sep;14(12):1389-96
38. Italiano A, Laurand A, Laroche A, et al. ERCC5/XPG, ERCC1, and BRCA1 gene status and clinical benefit of trabectedin in patients with soft tissue sarcoma. Cancer. 2011 Aug 1;117(15):3445-56
39. Sorokina LV, Pyatchanina TV, Didenko GV, et al. The influence of sodium dichloroacetate on the oxidative processes in sarcoma 37. Exp Oncol. 2011 Dec; 33(4):216-21
40. Liberti MV, Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci. 2016 Mar;41(3):211-218. doi: 10.1016/j.tibs.2015.12.001. Epub 2016 Jan 5. Erratum in: Trends Biochem Sci. 2016 Mar;41(3):287. Erratum in: Trends Biochem Sci. 2016 Mar;41(3):287
41. Rooke M, Coupland LA, Truong T, et al. Dichloroacetate is an effective treatment for sarcoma models in vitro and in vivo. Cancer Metab. 2014 May 28; 2(Suppl 1):P9
42. Zewde M, Kiyotani K, Park JH, et al. The era of immunogenomics/immunopharmacogenomics. J Hum Genet. 2018 Jul;63(8):865-875
43. Cantor JR, Sabatini DM. Cancer cell metabolism: one hallmark, many faces. Cancer Discov. 2012 Oct; 2(10):881-98
44. Miallot R, Galland F, Millet V, et al. Metabolic landscapes in sarcomas. J Hematol Oncol. 2021 Jul 22;14(1):114
45. Esperança-Martins M, Fernandes I, Soares do Brito J, et al. Sarcoma Metabolomics: Current Horizons and Future Perspectives. Cells. 2021 Jun 8;10(6):1432
46. Chadha M, Huang PH. Proteomic and Metabolomic Profiling in Soft Tissue Sarcomas. Curr Treat Options Oncol. 2022 Jan;23(1):78-88
47. Su M, Zhang Z, Zhou L, et al. Proteomics, Personalized Medicine and Cancer. Cancers (Basel). 2021 May 21;13(11):2512
48. Zou Z, Sun W, Xu Y, et al. Application of Multi-Omics Approach in Sarcomas: A Tool for Studying Mechanism, Biomarkers, and Therapeutic Targets. Front Oncol. 2022 Jul 8;12:946022
49. Perry LM, Cruz SM, Kleber KT, et al. Human soft tissue sarcomas harbor an intratumoral viral microbiome which is linked with natural killer cell infiltrate and prognosis. J Immunother Cancer. 2023 Jan;11(1):e004285
50. Narunsky-Haziza L, Sepich-Poore GD, Livyatan I, et al. Pan-cancer analyses reveal cancer-type-specific fungal ecologies and bacteriome interactions. Cell. 2022 Sep 29;185(20):3789-3806.e17
51. Cantwell AR Jr, Lawson JW. Necroscopic findings of pleomorphic, variably acid-fast bacteria in a fatal case of Kaposi's sarcoma. J Dermatol Surg Oncol. 1981 Nov;7(11):923-30
52. Li X, Saxena D. The tumor mycobiome: A paradigm shift in cancer pathogenesis. Cell. 2022 Sep 29; 185(20):3648–51