Animal models of well-differentiated/dedifferentiated liposarcoma: utility and limitations

Abstract: Liposarcoma is a malignant neoplasm of fat tissue. Well-differentiated and dedifferentiated liposarcoma (WDL/DDL) represent the two most clinically observed histotypes occurring in middle-aged to older adults, particularly within the retroperitoneum or extremities. WDL/DDL are thought to represent the broad spectrum of one disease, as they are both associated with the amplification in the chromosomal 12q13-15 region that causes MDM2 and CDK4 overexpression, the most useful predictor for liposarcoma diagnosis. In comparison to WDL, DDL contains additional genetic abnormalities, principally coamplifications of 1p32 and 6q23, that increase recurrence and metastatic rate. In this review, we discuss the xenograft and transgenic animal models generated for studying progression of WDL/DDL, highlighting utilities and pitfalls in such approaches that can facilitate or impede the development of new therapies.

Keywords: liposarcoma, transgenic animal model, xenograft

Introduction

Liposarcoma is an often fatal cancer of adipose tissue that accounts for approximately 20% of all adult soft tissue sarcomas.\(^1\) It can arise in almost any body district, although the most frequent sites are the extremities (24%) and the retroperitoneal region (45%), with a peak occurrence around the 5\(^{th}\) and 6\(^{th}\) decade and a slight predominance in males.\(^1\) Liposarcoma presents in 2 largest groups, indicated as well-differentiated liposarcoma (WDL) and dedifferentiated liposarcoma (DDL), in addition to the less frequent myxoid and pleomorphic subtypes. The diagnosis of each subtype is based on anatomical location, clinical behavior, histology appearance, and cytogenetic features.\(^2\)

Only WDL has no tendency to metastasize (unless it contains a dedifferentiated component) and may be therefore considered as a low-grade tumor, whilst the other subtypes show significant metastatic rates, ranging from 15% up to 50%.\(^2\) WDL/DDL histotypes share similar genetics despite a different prognosis and embody the most common cases observed clinically.\(^3\) WDL represents the largest group of malignant adipocytic neoplasms, accounting for approximately 40-45% of all cases.\(^2\) WDL is a slowly growing tumor distinguished by the presence of malignant adipocytes and spindle cells showing fibroblastic/myofibroblastic differentiation and giving rise to four subtypes, namely, adipocytic (or lipoma-like), sclerosing, inflammatory, and spindle cell variants. The most important prognostic factor for this tumor is anatomical location, which is also the main predictor of relapse. WDL arising at somatic soft tissue sites, such as limbs or the trunk wall, is alternatively termed atypical lipomatous tumor (ALT) since its complete surgical resection is usually curative. Instead, the term WDL...
is preferable used for tumors occurring in the retroperitoneum or other visceral sites, since the risk of local recurrences is about 50% and associated to a dedifferentiation process, causing an even higher mortality rate than that associated with metastasis. The term DDL was first introduced by Evans in 1979 to define the morphological progression from ALT/WDL to a non-lipogenic sarcoma. DDL indeed is considered a biphasic tumor consisting of a nonmetastatic WDL component juxtaposed to either a high-grade undifferentiated sarcoma with malignant fibrous histiocytoma or fibrosarcoma-like features or with a lower-grade sarcoma having the appearance of myxofibrosarcoma. DDL is more often recurrent, requires multi-organ resection more frequently and presents a shorter disease-free interval when compared to WDL. DDL variants are more predisposed to metastasize, while ALT/WDL subtypes do not metastasize without dedifferentiation. DDL behavior is peculiar showing also the tendency to develop heterologous myogenic (rhabdomyosarcomatous or leiomyosarcomatous), osteo/chondrosarcomatous, and rarely angiosarcomatous differentiation in approximately 5–10% of the cases. Both WDL and DDL are poorly responsive to conventional chemotherapy, and surgical resection represents the best management for operable disease. Adjuvant radiation is employed to reduce risk of local recurrence in case of high-grade DDL, whereas a first-line chemotherapy consisting of single-agent doxorubicin treatment is generally reserved for unresectable or metastatic diseases.

**Genomic landscape in liposarcoma**

Each liposarcoma subtype is characterized by a distinctive set of genetic signatures. Myxoid tumor type harbors the recurrent translocation t(12;16)(q13;p11) associated to the FUS-DDIT3 gene fusion product, whereas pleomorphic tumor is a complex-karyotype sarcoma frequently characterized by loss of TP53, RB1, and NF1. Nearby all WDL/DDL tumors are associated with the presence of one or more supernumerary circular (“ring”) and/or giant rod chromosomes. This leads to high-level amplifications in the chromosomal 12q13-15 region that causes overexpression of MDM2, the most observed amplification in WDL/DDL (close to 100%), and CDK4 (over 90% of the cases). The diagnostic detection of MDM2 and CDK4 by fluorescence in situ hybridization represents a reliable tool to discriminate WDL/DDL from other adipocytic tumors. Unsurprisingly, WDL shows high expression of genes associated with lipid metabolism and adipocytic differentiation, while DDL is characterized by upregulation of genes involved in proliferation and DNA repair as a result of additional genetic abnormalities, including losses, fusion transcripts, and amplifications.

Unlike WDL, DDL frequently contains 1p32, 6q23, and 12q amplifications causing oncogenic overexpression of AP-1, HMGA2, GLI1, MAP3K12, CDK2, ALX1, and TBX5. Over the last years, novel gene amplifications (UAP1, MIR557, LAMA4, CPM, IGF2, ERBB3, IGF1R), deletions at chromosome 1p (RUNX3, ARID1A), chromosome 11q (ATM, CHEK1), and chromosome 13q14.2 (MIR15A, MIR16-1), and recurrent mutations in members of PI3KCA, PTEN, WNT, ERBB, MAPK, and JAK-STAT pathways have been detected in DDL. Finally, an important role for epigenetic mechanisms in the dedifferentiation process is emerging, since CEBPα methylation was found in 24% of the DDL and CDKN2A gene promoter hypermethylation was observed in DDL but not in recurrent WDL.

The development of animal models recapitulating tumor progression, resistance, recurrence, and metastasis is vital for drug screening and biomarker analysis. Such approach includes two strategies, ie, the use of xenograft models and the development of transgenic models. Here we review the main liposarcoma models generated so far and discuss the advantages and limitations of such approaches.

**Xenograft models of liposarcoma**

The engraftment of human tumor cells into immunocompromised hosts (xenograft), despite being a limitation for studying the role of the immune system on tumor progression, is widely used to study cancer. For liposarcoma, this strategy represents the best option, given the difficulty to develop transgenic animal models (as highlighted in the next paragraph). As depicted in Figure 1, tumor samples obtained from surgical specimens are dissociated to single-cell suspensions for in vitro study or for cell-derived xenograft (CDX) injection into immunocompromised hosts. This can be done either under the skin (ectopic xenograft) or into the organ type in which the tumor originated (orthotopic xenograft). Orthotopic models ensure a more appropriate microenvironment but are more technically complex compared to ectopic models. A more personalized solution for patients with cancer is the use of tumor tissue fragments engrafted into immunocompromised mice. These patient-derived xenograft (PDX) tumors preserve the characteristics of the live tumor and better recapitulate tumor biology and intratumor
heterogeneity of patient tumors. The process of PDX generation for individualized care in advanced sarcoma has been set up: it takes 1 to 6 months, and approximately 75% of the implanted tumors grow successfully in mice. In this manner, while patients are receiving surgery and treatment with first-line therapy, the tumor is expanded across more generations of mice to test more appropriate treatments. The PDX models generated from surgical specimens maintain the tumor microenvironment present in the human host and the genetic features associated to intratumor heterogeneity, including gene expression profile, copy number variants, and treatment susceptibility. However, it should be advised that one recent study has reported that PDX models of varied tumor types develop mutations with serial passages that diverge from those observed in the patients. Recently, 5 PDX models were successfully established from surgical specimens and biopsies of 31 DDL patients. The tumors fragments were implanted bilaterally into the subcutaneous space of immunodeficient mice. Fragments from collected tumors were bilaterally re-implanted and passaged over multiple generations. Bilateral tumor engraftment and cryopreservation approaches of PDX models were used to reduce the number of mice required over time. Cryopreserved PDX tumors were successfully re-engrafted in mice. Such strategies are not avoid of limitations, including the need of a sufficient amount of fresh tumor tissue, the time of propagation and the failure rate of about 20% of the tumor implantation that, however, may predict lower aggressiveness. As reported in Table 1, several xenograft DDL models have been generated, while the engraftment of WDL lines is more difficult to obtain. In this regard, it has been proposed to keep the tumors inside a vascularized chamber during their growth into host mice to improve the engraftment success.

### MDM2/CDK4

As stated before, MDM2/CDK4 coamplification is the most observed genetic signature featuring WDL/DDL. The E3 ubiquitin ligase Mdm2 is a negative regulator of p53 tumor suppressor, whereas Cdk4 promotes cell cycle G1 phase progression through Rb protein phosphorylation. A CDX model was established to test a dual inhibitors strategy based on RG7388 and palbociclib compounds, inhibiting the p53-Mdm2 complex and Cdk4 activity, respectively. Over a 3-week treatment, the tumor volume was decreased and the progression-free survival was increased without evident toxic effects. In a PDX model established from a tumor specimen of a man presenting a high-grade DDL of the mesentery, genome sequencing showed MDM2/CDK4 coamplification and mutation on JAK2. Though, a limited clinical benefit was observed for the patient receiving Cdk4 inhibitor (P1446A-05) and further supplementation with Jak2 inhibitor (ruxolitinib). Since prospective drug sensitivity in the PDX model revealed the efficacy of ifosfamide treatment,
| Xenograft | Cell lines | Tumor drivers | Time to tumor formation | Treatment inhibitors | Treatment response | Ref. |
|-----------|------------|---------------|-------------------------|----------------------|--------------------|------|
| CDX       | Lipo246    | RTKs          | 10 days-6 months        | –                    | –                  | (37) |
|           | Lipo224    | MDM2/CDK4     | 2 weeks                 | RG7388/palbociclib co-treatment | Effective          | (44) |
|           | Lipo863    | P3K/Akt/mTOR  | 1 month                 | BEZ-235/RG7388 co-treatment | Effective          | (55) |
|           | IB115      | AXL           | 1 month                 | AXL knockout         | Effective          | (56) |
|           | IB115      |               |                         | EMDI214063           | Effective          | (59) |
|           | Lipo246    | MET           | 2 weeks                 | –                    | –                  | (63) |
|           | Lipo863    | IL-6          | 2 months                | miR-155 knockdown    | Effective          | (72) |
|           | Lipo246    | miR-155       | 3–6 weeks               | –                    | –                  |      |
| PDX       |            | MDM2/CDK4/JAK2 mutations | 2–6 months | P1446A-05/ruxolitinib co-treatment | Moderately effective | (34) |
|           |            | MDM2          | 2–4 months              | ifosfamide           | Effective          | (36) |
|           |            | MDM2/RTKs     | 1–4 months              | –                    | Effective          | (45) |
|           |            | MDM2          | 2 months                | pazopanib            | Effective          | (46) |
|           |            | P3K/Akt/mTOR  | 6 months                | ALGP-doxo            | More effective compared to doxo | (50) |
|           |            | miR-193b      | Unreported              | sorafenib/rapamycin co-treatment | Effective          | (73) |
|           |            |               |                         | miR-193b injection   | Effective          |      |
|           |            |               |                         | PF-271               | Effective          |      |

Abbreviations: DDL, dedifferentiated liposarcoma; CDX, cell-derived xenograft; PDX, patient-derived xenograft; RTKs, tyrosine kinase receptors.
this revealed a good efficacy in the patient. In 5 PDX models generated from surgical specimens of 31 DDL patients, FISH analysis revealed MDM2 amplification throughout passages. Some of these PDX models have already been successfully used for in vivo testing of a tyrosine kinase inhibitor (pazopanib) and a cytotoxic prodrug PhAC-ALGP-doxorubicin (ALGP-doxo). In two bilaterally transplanted PDX models, treatment with ALGP-doxo, that is converted to doxorubicin by peptidases present in tumor cells and/or tumor microenvironment, showed a significantly higher antiproliferative effect compared to doxorubicin.

RTKs and downstream pathways

WDL/DDL show high expression of several RTKs, including DDR2, ERBB3, NTRK1, FGFR3, ROS1, MET, AXL, KIT, and IGFR. This leads to high activation of downstream signaling primarily through the Mapk and PI3K/Akt/mTOR pathways. The overactivation of Akt pathway in WDL/DDL frequently occurs because of the loss of PTEN or the presence of activating mutations in the PI3K gene (E542K and H1047R amino acid substitutions). This pathway, eliciting protein synthesis via mTOR, supports many cellular functions, including growth, metabolism, and survival. Moreover, oncogenic signal transduction through the PI3K-Akt pathway can enhance Mdm2-mediated p53 suppression. In PDX models, treatment with a tyrosine kinase inhibitor (pazopanib) and an mTOR inhibitor (rapamycin) yielded a reduction of tumor growth that was more consistent compared to rapamycin treatment alone. In CDX tumors, concomitant inhibition of the PI3K/Akt/mTOR and Mdm2 pathway, mediated by BEZ-235 and RG7388 compounds, promoted a significant reduction of tumor growth. Reduced tumor growth and metastatic rate of CDX tumors were also reported upon knockdown of AXL, a member of the TAM family that signals through PI3K/Akt/mTOR and Mapk pathways and whose inhibition represents a promising avenue for the treatment of a wide number of cancers. Another emerging therapeutic target is the Met receptor, highly expressed in liposarcoma. After binding to Hgf ligand, Met receptor transactivates Stat3, SFKs, and Mapk pathways. Consistent with this, treatment with a novel Met inhibitor (EMD1214063) was reported to abrogate tumor growth in CDX models.

IL-6

IL-6 is a cytokine often overexpressed in cancer and associated to a poor prognosis and chemoresistance. IL-6 expression is under the control of a number of transcription factors including NF-κB activator, AP-1, and CEBPs. Especially in adipose tissue, CEBPs play a pivotal role as they regulate several biological responses like proliferation, differentiation, adipocytes maturation, and cytokines production. To test the potential role of IL-6 on liposarcoma pathogenesis, DDL cells were intramuscularly injected into nude mice. Tumor growth was then monitored in both voluntary-active or inactive mice with open or restricted access to activity-wheels to test the potential effects of physical activity on tumor progression. The authors found a greater amount of the circulating IL-6 (6-fold increase) in tumor-bearing mice that was correlated with CEBP-α/β and Ppar-γ activities in comparison to controls. Of note, in mice subjected to a physical activity the levels of IL-6 were enhanced, inducing tumor growth, body weight loss, and lung metastasis dissemination through the activation of the autophagy program. The negative effect of regular physical activity was then confirmed using an orthotopic tumor model characterized by intramuscular tumor growth, indicating that patients with lower-extremities liposarcoma could be advised to minimize the physical activity during the preoperative period.

MicroRNA signatures

MicroRNAs (miRs) are evolutionarily conserved noncoding small RNAs of 18- to 24-nucleotides involved in post-transcriptional gene expression regulation through mRNA degradation, translational inhibition, or chromatin-based silencing mechanisms. The miR expression profiles are markedly altered in cancer and their signatures in human tumors are associated with diagnosis, staging, progression, prognosis, and response to treatment. Early detection of recurrent or metastatic disease through miR predictors could improve patient prognosis. MiR signatures that are unique to liposarcoma subtypes have been proposed. For example, miR-155 upregulation was detected in all liposarcoma tumors and its plasma levels have been reported as a diagnostic marker for DDL. On the other side, miR-25-3p and
miR-92a-3p are secreted by liposarcoma cells through extracellular vesicles and may be useful as potential disease biomarkers.71 Using a miR array platform, an expression signature consisting of 4 overexpressed and 31 downregulated miRs was found to differentiate WDL/DDL from normal fat.72 The most statistically significant upregulated miR in DDL was confirmed to be miR-155, which promoted tumor cell growth by targeting CK1α that led to increased β-catenin signaling and cyclin D1 expression.72 Consistent with these results, miR-155 knockdown in CDX tumors delayed tumor growth.72 MiR-193b has lower expression in WDL/DDL compared to adipose tissue samples73 and its injection into PDX tumors significantly delayed tumor volume only after 3 weeks of treatment.73 Since miR-193b was found to target the FAK proteins, the tumor treatment with a FAK inhibitor (PF-271) reduced tumor growth via increased cell apoptosis.73

Transgenic animal models of liposarcoma

Transgenic animal models are mainly originated through genetic knock-in or knock-out approaches to express and/or inactivate specific genes in a tissue-specific and time-dependent manner. The procedures for their generation are expensive and time consuming; however, the availability of animal models developing tumor in response to genomic alterations is of great help for scientists and clinicians. Given the rarity of liposarcoma, the number of transgenic animal models developed so far is limited. Perhaps, one of the major difficulties in such approach is represented by the choice of the cell precursor in which the genetic lesions have to be introduced. In this context, it has been proposed that WDL/DDL may share a common ancestral cell of origin and that the gradual accumulation of genetic lesions could drive the progression of WDL to DDL.74 Alternatively, both WDL/DDL could arise from the same cell precursor but at different time points along the multistep process of adipogenic differentiation.75 Since approximately 5% cases of DDL show heterologous cellular composition of myogenic cells (leiomyosarcoma or rhabdomyosarcoma),8 potential candidate cells are the multipotent stem cells that can differentiate into different mesenchymal precursors. Furthermore, it must be mentioned that cell transdifferentiation processes are possible, as it has been observed that the aberrant activation of the Shh signaling in mature adipocytes is sufficient to convert them into myogenic tumor cells.76 As depicted in Figure 2, four transgenic animal models developing liposarcoma have been generated through gene manipulation in mesenchymal cell progenitors or adipocytes, as described below.

Akt signaling

Oncogenic gene mutations in receptors (FGFR, EGFR) or transducers (KRAS, PI3K)77 commonly elicit deregulation of PI3K/Akt/mTOR pathway, as analogously observed in liposarcoma.28,29 A transgenic model of WDL has been generated via targeted expression of an active myristoylated Akt2 form in mesenchymal cell progenitors of zebrafish carrying p53 homozygous mutation. Following embryo microinjection with a DNA construct carrying Akt2,49 solid tumors classified as WDL (91%) and osteosarcomas (9%) developed between 1 and 4 months of age, with the highest tumor incidence rate observed in p53 homozygous mutants (about 29%) (Figure 2A). The treatment of transgenic zebrafish with a dual PI3K/mTOR inhibitor (BEZ235) was efficacious to counteract tumor growth via increased apoptosis,49 therefore confirming that this pathway synergizes with p53 loss to drive liposarcoma genesis.

Notch signaling

The evolutionarily conserved Notch signaling pathway plays a pivotal role in cell commitment, tissue development, and tumorigenesis.78,79 The Notch cascade is initiated when one of the five ligands belonging to DSL family (Jag1, −2 and Dll1, −3, −4) binds to one of the four Notch receptors.80 This interaction induces sequential cleavages in Notch receptor mediated by different proteases (Adam metalloproteases, γ-secretase protease complex) that cause the release of the Notch intracellular domain, which then translocates into the nucleus to activate the transcription of several target genes.80 The activation of Notch signaling in mature adipocytes, obtained through the Cre/LoxP technology,81 has been shown to elicit DDL formation by shaping gene expression and promoting dedifferentiation via suppression of fatty acid metabolism and Ppar-γ function (Figure 2B).82 Mice with activated Notch1 exhibited a lipodystrophy phenotype in the preneoplastic state, characterized by hepatic steatosis, hyperglycemia, and severe insulin resistance.82 The authors further demonstrated that the treatment of a human LPS246 xenograft model with a Notch inhibitor (dibenzazepine) was effective to reduce tumor growth.82 A selective Notch inhibitor (LY3039478)
was recently tested in a phase 1a/b trial showing a modest clinical activity and a safety profile towards several sarcomas, including liposarcoma. Interestingly, it has been shown that Mdm2 can synergize with Notch1 to inhibit apoptosis and promote proliferation, indicating that targeting the Notch pathway may be helpful for overcoming WDL/DDL development and progression.

**IL-22 overexpression**

WDL formation has been observed in transgenic mouse overexpressing IL-22 in adipocytes (Figure 2C). Interestingly,
these mice had neither apparent phenotype nor metabolic alteration, but developed spontaneous tumors in adipose tissue after long-term feeding with high fat diet. IL-22 is a T-cell secreted cytokine that modulates inflammatory response in nonhematopoietic tissues such as epithelium and liver via primary activation of Jak1 and Tyk2 and Stat3 pathway but also of Mapk and PI3K/Akt/mTOR pathways. The oncogenic role of IL-22/Stat3 signaling axis has been reported in a number of tumors, such as hepatocarcinoma. In response to high IL-22 levels, the adipose tissue of transgenic mice became inflamed and characterized by higher levels of IL-1β, IL-6, IL-10, TNF-α, and Erk pathway activation. This work confirms the important role of diet and inflammation in tumorigenesis, since the increased secretion of steroid hormones and insulin resistance in the presence of a persistent inflammatory state, as observed in obesity, may increase tumorigenesis risk. It has been estimated that obesity in children from 2 to 14 years resulted associated with increased cancer risk in adulthood by 40% and also with a worse survival with respect to control patients.

### Epistatic gene interaction between the adipose triglyceride lipase and hormone-sensitive lipase

Epistasis refers to the observation of an unexpected phenotypic outcome when combining two genetic alleles. A synergistic epistatic interaction determinant for liposarcoma development has been observed between two genes of the lipolysis pathway, the adipose triglyceride lipase (Atgl) and the hormone-sensitive lipase (Hsl) (Figure 2D). Lipolysis has been implicated in cancer since it provides a source of fatty acids for tumor growth. Atgl enzyme, encoded by the PNPLA2 gene, catalyzes the hydrolysis of triglycerides to diglycerides, whereas Hsl, encoded by the LIPE gene, catalyzes the second step of lipolysis, the cleavage of diglycerides to monoglycerides. Of note, deletion of PNPLA2 is reported in WDL and sarcoma, and deletions of the chromosome 19p13 region containing LIPE are frequent in DDL and correlate with poor prognosis. Mice lacking both Atgl and Hsl showed near-complete deficiency of lipolysis and were unable to maintain their blood glucose values over a normal postprandial fasting due to rapid depletion of carbohydrates reserves in the absence of lipid stores in adipose tissue. While no malignant tumors were found in white adipose tissue of transgenic mice, the brown adipose tissue was characterized by hypertrophic brown adipocytes with formation of liposarcoma tumors between 11 and 14 months of age. Expression profiling analysis in premalignant brown adipose tissue of transgenic mice revealed downregulation for the gene sets of fatty acid, triacylglycerol and ketone body metabolism, the tricarboxylic acid cycle and respiratory chain and genes of lipid metabolism. In contrast, genes involved in the immune response were upregulated. Among the differentially expressed genes, liposarcoma tumors showed highest expression of GPNMB, which encodes a circulating glycoprotein identified in several cancers, and lowest expression of G0S2, the endogenous inhibitor of Atgl. By comparing the gene expression profiles between transgenic animals and a number of 58 DDL patients, the authors found that LIPE, PNPLA2, and G0S2 are also among the five most downregulated genes in human liposarcoma.

### Conclusions

The generation of animal models of liposarcoma is crucial for identification of early markers, diagnosis, and development of new therapies. To date, a major obstacle in this process is the limited number of appropriate animal models recapitulating the complexity and heterogeneity of liposarcoma malignancies, resulting in poor efficiency in translating the findings of basic research to clinical applications. Surely, the generation of animal models is complicated by the choice of the potential cell of origin to be used as a recipient for the genomic editing phase. In this context, the use of PDX models allows to personalize the treatment options for patients and therefore represents an important alternative. Hopefully, the establishment of novel clinically relevant disease transgenic models will be vital for identification of the molecular mechanisms underlying liposarcoma genesis and progression and for validation of new therapies.

### Abbreviation list

- **ADAM**: A disintegrin and metalloprotease
- **AKT**: Ak strain transforming murine thymoma viral oncogene
- **ALK**: Alkistant-like homeobox protein 1
- **AP-1**: Activator protein 1
- **ARID1A**: AT-rich interactive domain 1A
- **ATM**: Ataxia-telangiectasia-mutated gene
- **CDK**: Cyclin-dependent kinase
- **CDKN2A**: Cyclin-dependent kinase inhibitor 2A
- **CEBP**: CCAAT/enhancer-binding proteins
- **CHEK1**: Checkpoint kinase 1
- **CK1α**: Casein kinase 1 alpha
- **CPM**: Carboxypeptidase M
- **DDR2**: Discoidin domain receptor 2
- **DLL**: Distal-less
- **DSL**: Delta/serrate/lag-2
- **EGFR**: Epidermal growth factor receptor
- **ERbB**: Erb-b2 receptor tyrosine kinase
- **ERK**: Extracellular signal-regulated kinase
- **FAK**: Focal adhesion kinase
- **FGFR**: Fibroblast growth
factor receptor; FUS-DDIT3, Fusion-DNA damage-inducible transcript 3; G0S2, G0/G1 switch gene 2; GLI1, Glioma-associated oncogene homolog 1; GPNNMB, Glycoprotein nonmetastatic melanoma protein B; HGF, Hepatocyte growth factor; HMGAA2, High-mobility group AT-hook protein 2; IL, Interleukin; IGF2, Insulin-like growth factor 2; IGFR, Insulin-like growth factor receptor; JAG1, Jagged-1; JAK, Janus kinase; KIT, receptor tyrosine kinase; Kras, Kirsten rat sarcoma viral oncogene homolog; LAMA4, Laminin subunit alpha 4; LIPE, Lipase E; MAPK, Mitogen-activated protein kinase; MDM2, Mouse double minute 2 homolog; MET, Mesenchymal-epithelial transition factor; mTOR, Mammalian target of rapamycin; NF1, Neurofibromin 1; NF-kB, Nuclear factor kappa B; NOTCH1, Neurogenic locus notch homolog protein 1; NTRK1, Neurotrophic tyrosine receptor kinase type 1; PI3KCA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PNPLA2, Patatin-like phospholipase domain containing 2; PPAR-γ, Peroxisome proliferator-activated receptor gamma; PTEN, Phosphatase and tensin homolog; RB1, Retinoblastoma 1; ROS1, C-roS oncogene 1; RTKs, Tyrosine kinase receptors; RUNX3, Runt-related transcription factor 3; SFKs, Src family protein kinases; SHH, Sonic hedgehog; STAT, Signal transducer and activator of transcription; TAM, Tyro3, Axl, MerTK; TBX5, T-box transcription factor 5; TNF-α, Tumor necrosis factor-alpha; TP53, Tumor suppressor protein p53; TYK2, Tyrosine kinase 2; UAP1, UDP-N-acetylglucosamine pyrophosphorylase 1; WNT, Wingless-integrated.

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Author contributions
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