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Review Article

A review on molecular mechanisms underlying the contribution of Tspan8 and CD151 tumor and host exosomes to tumor progression, hematopoiesis and angiogenesis

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Abstract

Tetraspanins associate with many proteins and are involved in numerous activities like the crosstalk between cells and matrix, tumor progression, angiogenesis, and hematopoiesis. These multiple activities are mostly executed via exosomes (Exo) or tumor-derived Exo (TEX) and are impaired in Tspan8-knockout (ko), CD151ko and Tspan8ko/CD151ko (dbko) mice and cells. Strongly impaired tumor cell dissemination RTK with limited migration and invasion relied on distorted associations with cell adhesion molecules and missing protease recruitment and was rescued by coculture with wild type (wt) Exo. Defects in early hematopoietic progenitor maturation depended on the failed association of hematopoietic growth factor receptors with CD151 and Tspan8. Constrained angiogenesis in ko mice was due to the failure of recruiting GPCR via Tspan8 and CD151. It was rescued by wt Exo. An additive impact of Tspan8ko and CD151ko in dbko cells depended on differences in preferred associations of Tspan8 and CD151. All defects relied on reduced delivery as well as uptake of Exo and TEX by ko cells. Here we did a literature search on the underlying mechanisms.

Keywords: Tspan8 knockout, CD151 knockout, Exosomes, TEX, Metastasis, Angiogenesis, Hematopoiesis

List of Abbreviations: AA: Amino Acid; A2BR: Adenosine Receptor 2b; AB: Antibody; AD: Alzheimer's Disease; ADAM: Disintegrin-Metalloproteinases; ADO1: Adenosine; AGE: Advanced Glycation End product; AGM: Yolk sac, the paraaortic splanchnopleura/aorta, gonad, mesonephros region; AGSA: Activator of G-protein Signaling 8; Akap12: A-kinase anchor protein 12; AKT: Serine/Threonine Kinase; ALDHA1: Aldehyde Dehydrogenase 1 family, member A1; Ang: Angiopoietin; AP2a: Activating Enhancer-Binding Protein 2α; ApoE: Apolipoprotein E; AR: Androgen Receptor; AR-V7: Splice Variant of AR; Arg: Arginase, catalyzes arginine into ornithine + urea; ARID4B: AT-rich Interaction Domain 4B; α-SMA: Alpha-Smooth Muscle Actin; ATXN3: Ataxin 3; β-cat: β-catenin; b-Raf: Proto-oncogene B-Raf; BBB: Blood-Brain Barrier; BC: Breast Cancer; BM: Bone Marrow; BMC: BM Cells; BMP: Bone Morphogenetic Proteins; BMSC: BM Stem Cell; Cadh: Cadherin; CAF: Cancer Associated Fibroblast; Casp: Caspase; cat: β-catenin; Cav: Caveolin; CBF: Cerebral Blood Flow; CC: Cervical Cancer; c-Cbl: E3 ubiquitin-protein ligase Cbl; c-coagp: Consumption coagulopathy; CCR: C-C Chemokine Receptor type; CD56: Neural Cell Adhesion Molecule; CD62L: L-selectin; CD105: Endoglin; CD106: Vascular Cell Adhesion Molecule 1; CD137: Tumor Necrosis Factor Receptor Superfamily Member 9; CD138: Syndecan 1, a Heparin Sulfate Glycoprotein; CD146: Cell Surface Glycoprotein MUC18; CD151-ARSA: YRSL mutation of CD151; CDC42: Cell Division Control Protein 42 Homolog; CLA: Cutaneous Lymphocyte Antigen; Cldn: Claudin; CLP: Cyclo L-Leucyl-L-Prolyl peptide; CM:

Conditioned Medium; CNTN1: Contactin1; CNV: Copy Number Variations; COPB2: Coatomer Protein Complex Subunit Beta 2; CRC: Colorectal Cancer; CSC: Cancer Stem Cells; c-Src: Tyrosinkinase Src (cellular sarcoma); ctn: Catenin; CXCL: C-X-C Motif Chemokine Ligand; CXCR3: CXC-Motiv-Chemokinrezeptor 3, CD183; CXCR4: CXC-Motiv-Chemokinrezeptor 4, CD184, Stromal cell-derived factor 1 receptor; Cu: Copper; d: Day; dbko: Double knockout; DEX: Dexamethason; Dia1: Diaphanous-related formin-1; DLEC: Dermal LEC; DLL4: Delta like Canonical Notch Ligand 4; EC: Endothelial Cells; E-cadh: E-cadherin; ECFC: Endothelial Colony Forming Cell; ECM: Extracellular Matrix; EE: Early Endosomes; EFNB2: EphrinB2; EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; EMT: Epithelial-Mesemchymal Transition; Endo: Endothelin; eNOS: G-protein-Coupled Receptors (GPCR); EPC: Endothelial Progenitor Cells; EPCAM: Epithelial Cell Adhesion Molecule; EphA4: EPH Receptor A4; EPO: Erythropoietin; EPOR: EPO Receptor; ErbB2/HER2: Erb-B2 Receptor Tyrosine Kinase 2; ERK1/2: Extracellular Signal Regulated Kinases; ERM: Ezrin/radixin/moesin; Exo: Exosomes; EV: Extracellular Vesicles; FAK: Focal Adhesion Kinase; FB: Fibroblast; FGF: Fibroblast Growth Factor; FGFR: FGF Receptors; FLOT: Flotillin; FN: Fibronectin; FOXL2: Forkhead Box Protein L2; FoxM1: Forkhead Box Protein M; FOXO3: Forkhead Box O3; FoxP1: Forkhead Box Protein P1; FLT3: Receptor-Type Tyrosine-Protein Kinase FLT3; FU: 5-Fluorouracil; G12/13: Alpha Subunits Of Heterotrimeric G Proteins Linking GPCR to Guanine Nucleotide Exchange Factors; Gab2: GRB2-Associated-Binding Protein 2; GATA2/6: GATA-Binding Factor 2/6; GC: Gastric Cancer; G-CSF: Colony-Stimulating Factor; GM1: Monosialotetrahexosylganglioside; GPCR: G-protein Coupled Receptor; Grb2: Growth Factor Receptor-Bound Protein 2; Grb2-Gab1: Growth Factor Receptor Binder 2 (Grb2)-Grb2-Associated Binder (Gab)1; GRF: Growth Factor Receptors; GSK-3β: Glycogen Synthase Kinase-3β; H: Hour; HCC: Hepato Cellular Carcinoma; HEL: Immortalized Cell Line of Acute Erythroid Leukemia; HEP-3: Hepatoma Line; Her2: Human Epidermal Growth Factor Receptor 2 = ERBB2; HF: Heart Failure; HGF: Hepatocyte Growth Factor; HDAC: Histone Deacetylase; hPMEC: Placental Microvascular EC; HSC: Hematopoietic Stem Cells; HSPC: Hematopoietic Stem and Progenitor Cell; HUVEC: Human Umbilical Vein Endothelial Cells; IGFR: Insulin Growth Factor Receptor; Ifp: IntrafoodpadIL: Interleukin; ILK: Integrin-Linked Kinase; IQGAP1: Ras GTPase-Activating-Like Protein IQGAP1; IR: Ionizing Radiation; JAG1: Jagged 1; JAK: Janus Kinase; K36: Histone H3 at Lysine 36; Kd: Knockdown; Ko: Knockout; KIT: Proto-oncogene c-KIT, CD117; LC: Lung Adenocarcinoma; LCMR1: MHC class I-Related Protein; LEC: Lymphatic EC; LEL: Large Extracellular Domain Of Tetraspanins; LGR5: Leucine-Rich Repeat-Containing G-protein Coupled Receptor 5; LIF: Leukemia Inhibitory Factor; LN: Lymph Node; LV: Lymphatic Valve; LVV: Lymphovenous Valves; Lyn: Tyrosine-Protein Kinase Lyn; LPAR2: LPA Receptor 2; mAb: Monoclonal AB; MAPK: Mitogen-Activated Protein Kinases; MaSC: Mammary SC; MCA: Methylcholanthrene; MCF-10A: Mammary Epithelial Cancer Cell Line; mCRC: Metastatic CRC; MDSC: Myeloid-Derived Suppressor Cells; MEK: Mitogen-Activated Protein Kinase Kinase = MAP2K = MAPKK; MET: Hepatocyte Growth Factor (HGF) Receptor; MHC: Major Histocompatibility Complex; MIF: Macrophage Migration Inhibitor Factor; MM: Multiple Myeloma; MMP: Matrixmetalloprotease; MMTV: Mouse Mammary Tumor Virus; MPP: Hematopoietic Multipotent Progenitor; MSC: Mesenchymal Stroma Cells; mTOR: Mammalian Target of Rapamycin; MT1-MMP: Membrane Type 1-Matrixmetalloprotease; MUC1: Glycoprotein with Extensive O-linked Glycosylation; MYC: Myc protooncogene; NANOG: Homeobox Transcription Factor Nanog; N-cadh: N-cadherin; NCSC: Non-Cancer Stem Cells; NFκB: Nuclear Factor-κB; NSG: NOD Scid Gamma; NO: Nitric Oxide; NPC: Nasopharyngeal Carcinoma; NRF2: Nuclear Factor Erythroid 2-Related Factor 2; NRP: Neuropilin; OC: Ovarian Cancer; OCT4: Octamer-Binding Transcription Factor 4 = POU5F1; OE: Overexpression; OS: Osteosarcoma; OXPHOS: Oxidative Phosphorylation; p38: p38 Mitogen-Activated Protein Kinases; p65: TF p65 or Nuclear Factor NF-kappa-B p65 subunit; p120cnt; p130Cas: p130 Crk-Associated Substrate; p130Crk: Retinoblastoma-Related p130 Pocked Protein; PB: Phenylbutyrate; PC: Plasma cells; PCL: Pyrocatechol; PDAC: Pancreatic Adenocarcinoma; PDGF: Plateled Derived Growth Factor-BB; PDGFRA /B: PDGF Receptors; PDNP: Podoplanin; PDZ-RhoGEF: Rho Guanine Nucleotide Exchange Factor 11; p-GSK-3β: Glycogen Synthase Kinase-3 beta; PI3K: Phosphoinositol 3 Kinase; PKC: Protein Kinase C; PL: Platelets; PLC: Pyrocatechol; pMSC: Placental Mesenchymal Stem Cells; PrCa: Prostate Ca; PRDX2: Peroxiredoxin 2; Prox1: Prospero Homeobox Protein 1; PTC: Papillary Thryroid Carcinoma; PTCH1: Protein Patched Homolog 1; PVT1: lncRNA; R1: Notoginsenoside R1; ras: Rat Sarcoma Virus Small GTPase; rac: Subfamily of the Rho family of GTPases; raf: Rapidly Accelerated Fibrosarcoma; rapa2: Ras Family GTPase; rabGAP: Rab GTPase Activating Protein; RC: Rectal Carcinoma; RGD: Arg-Gly-Asp; Rho GTPases: Subfamily of Small G-proteins of the Ras Superfamily; Rictor: Rapamycin-insensitive Companion of Mammalian Target of Rapamycin; RT: Radiation Therapy; RTK: Receptor Tyrosine Kinase; SB431542: Drug Candidate as an Inhibitor of ALK Receptors; Sc: Subcutaneous; SCF: Stem Cell Factor; SCI: Hemisection Spinal Cord Injury; SDF-1: Stromal Cell-Derived Factor-1; SENP1: SUMO Endopeptidase; PAI-1: Plasminogen Activator Inhibitor Serpin E1; Sentrin: Sentrin/SUMO-Specific Protease; sExo: Serum Exo from Tumor Free Mice; SF: Scatter Factor / Identical to HGF; SGC: Salivary Gland Cancer; SHH: Sonic Hedgehog; sh2: Src Homology 2; shp2: Src Homology Region 2 domain-Containing Phosphatase-2; SENP6: SUMO-Specific Protease 6; SLC31A1: High Affinity Copper Uptake Protein 1; Slug: Zinc Finger Protein SNAI2; SMAD: Small Mother Against Decapentaplegic; SMCC: Small-Molecule Compounds Cocktail (DAPT, DEX, SB431542, ascorbic acid); SMO: Sequential Minimal Optimization; SOX18: TF SOX18 (SRY-Related HMG Box (SOX) Transcription Factor); SOX21-AS1: SRY-Box Transcription Factor 21 Antisense Divergent Transcript 1 lncRNA; src: Proto-oncogene Protein Tyrosine Kinase; SSC: Spermatogonial SC; SSH: Suppression Subtractive Hybridization; STAT: Signal Transducer And Activator Of Transcription; STS1/STS2: Space Transportation System-1/2; SVG: Salivary Gland Cancer; TAU: The Antiviral Drug 2-Thio-6-Aza-Uridine; TAZ: Tafazzinfamily Protein; TBC1D10: TBC1 Domain Family Member 10A; TBI: Traumatic Brain Injury; TCF/LEF: T cell Factor/Lymphoid Enhancer Factor Family; TEM: Tetraspanin-Enriched Membrane Microdomain; TEX: Tumor Cell Derived Exosones; TF: Transcription Factor; TFGRI, TGFbRII: Transforming Growth Factor Receptor I / II; TfR: Transferin Receptor; TGF-β1: Transforming Growth Factor; ThbSp: Thrombospondin; Tie2: TEK Tyrosine Kinase, CD202B; TIK: Bcr-Abl Tyrosine-Kinase Inhibitor; TME: Tumor Microenvironment; TNBC: Triple-Negative Breast Cancer; TNC: Tenascin; TNF: Tumor Necrosis Factor; TNIK: TRAF2 and NCK Interacting Protease; TSG101: Tumor Susceptibility Gene 101; Tspan8: Tsp8; uPAR: Urokinase Plasminogen Activator Receptor; USP11: Ubiquitin Carboxyl-Terminal Hydrolase; UTI: Ulinastatin, Urinary Trypsin Inhibitor; VCAM1: Vascular Cell Adhesion Molecule-1; VEC: Vascular Endothelial Cells; VEGF: Vascular Endothelial Growth Factor; VEGFR: VEGF Receptor; vim: Vimentin; VTN: Vitronectin; vWf : Von Willebrand factor; WB: Western blot; wk: Week; Wnt1: Proto-oncogene Wnt-1; wt: Wild type; YAP: Yes-Associated Protein 1

Introduction

Exosomes (Exo) or small extracellular vesicles are delivered by many non-transformed cells (sExo) and abundantly by tumor cells (TEX) [1,2]. They are composed of a lipid bilayer with incorporated and attached membrane and cytosolic molecules, coding and noncoding RNA [3,4]. sExo/TEX distribute through the body, selectively bind to or fuse with target cells [5,6]. All sExo/TEX components being function competent [6,7], binding/uptake is accompanied by target modulation [6–9]. Importantly, cells deliver several types of sExo/TEX that differ by early endosome (EE) origin from cytoplasmic organelles or invaginated membrane domains like caveolae, clathrin-coated pits or tetraspanin-enriched microdomains (TEM) [10–12]. The latter are characterized, besides others, by distinct lipid composition [13,14]. Finally, tetraspanins are consistently enriched in sExo/TEX and contribute to target selection [14,15]. However, their path towards sExo/TEX is still disputed [16].

Tetraspanins are highly conserved 4-transmembrane proteins with a small and a large extracellular loop [17]. The latter accounts for dimerization and association with non-tetraspanin transmembrane and attached cytosolic molecules [18]. The lipid composition of TEM-derived sExo/TEX differs from that of non-TEM-derived Exo, where monosialotetrahexosylganglioside (GM1) is suggested contributing to TEM-derived tetraspanin stabilization in light density membrane fractions [14,19–22]. This aspect is utmost important, as only TEM-derived sExo/TEX are recovered in light density fractions. In view of the high expression of CD9, recovery in light density fractions was the only possibility to differentiate from CD9-mediated effects, CD9 not being recovered in light density fractions. Finally, tetraspanins mostly act as molecular facilitators via associated proteins [23], it is important to note that TEM-derived Tspan8 and CD151 complexes may not become incorporated into multivesicular bodies. Instead, they can migrate through the cytoplasm without taking up additional cytoplasmic components and are released unaltered as sExo/TEX [24]. This facilitates the assignment of functional differences in ko cells and sExo/TEX to CD151, respectively, Tspan8.

We will discuss in detail signaling by the association of Tspan8 and CD151 with adhesion molecules [25,26], with proteases [27–29], signaling explaining differences between Tspan8ko and CD151ko cells [30–32], signaling by the association with receptor tyrosine kinases (RTK) and G-protein coupled receptor (GPCR) [33–36]. Finally, a possible involvement of metastasis associated molecules should be mentioned [37].

In advance of discussing possible signaling pathways, we want to state that we attach in the supplement the long list of abbreviations. This should facilitate reading without continuously shifting to the list of abbreviations.

Discussion

Section 1. The special role of Tspan8 and CD151 in exo biogenesis and delivery

To unravel the impact of Tspan8- and CD151-competent sExo/TEX some special features of their biogenesis and delivery should be outlined.

1. Tspan8 and CD151 are located in special microdomains called TEM, with a selective lipid composition. Accordingly, they are exclusively recovered in light density fractions [13,14].

2. TEM-derived early endosome (EE) can travel through the cytoplasm bypassing multivesicular bodies that implies sExo/TEX composition corresponds to that of the invaginated TEM [24].

3. sExo/TEX delivery is reduced in Tspan8ko, CD151ko and double (db)ko cells [30–32]. This is utmost surprising taking into account the multiple pathways of membrane invagination / EE generation, where TEM-derived EE account for a negligible minority.

4. Tsp8 and CD151 are associated with a multitude of molecules that cover adhesion molecules, proteases, receptor tyrosine kinases (RTK), G protein coupled receptors (GPCR) and associated intracellular signaling molecules. As Tspan8- and CD151-associated molecules differ, the recovery in sExo/TEX also differs [30–32].

5. The multitude of Tsp8- and CD151-associated molecules of the TEM complexes of sExo/TEX will find multiple ligands on non-transformed and transformed cells and the extracellular matrix (ECM) facilitating strong attachment, persistence as well as fusion with the cell membrane or penetration and incorporation into the host cell [5,6,14,15,30,38–42]. Reduced dbko sExo/TEX uptake also accounts for endothelial cells (EC) and bone marrow cells (BMC) (please see below).

6. Finally, although tetraspanin webs of sExo/TEX dominate uptake, Tspan8 and CD151 expression on the acceptor cell also influences sExo/TEX uptake such that uptake of TEX by Tspan8ko and/or CD151ko tumor cells is impaired; sExo uptake by Tspan8ko and/or CD151ko EC is impaired and Tspan8ko and/or CD151ko BMC display reduced sExo/TEX uptake.

In detail: First to note, expression of classical exosome markers (CD63, CD81, Alix, Lamp1, TSG101, CD40d, α6, CD41, CD61, ThbSp, CD47 and major histocompatibility complex (MHC) I were unaltered in sExo of wt and ko mice. Solely CD151 was absent in sExo/TEX of CD151ko and dbko mice and Tsp8 was absent in sExo/TEX of Tspan8ko and dbko mice. Additionally, neural cell adhesion molecule (CD56) and vascular cell adhesion molecule 1 (CD106) recovery was reduced in CD151ko and dbko sExo. Recovery of TEX was evaluated in tumor cell culture supernatant. Delivery of TEX was reduced in Tspan8ko (~40%), CD151ko (~25%) and dbko (~55%) tumor cells. Delivery of sExo in tumor-free mice was reduced in Tspan8ko (~35%), CD151ko (~25%) and dbko (~40%). Due to their importance the data are summarized presenting the % tumor cells and TEX stained by anti-CD63, -CD81, -CD151 and -Tspan8. CD63 and CD81 are expressed by ~30% of tumor cells and ~35% of TEX. CD151 is expressed by ~30% of wild type (wt) and Tspan8ko tumor cells, but by ~60% of wt and Tspan8ko TEX. Similarly, Tspan8 is expressed by ~15% of wt and CD151ko tumor cells, but by ~46% of wt and CD151ko TEX. We interpret the striking increase only of CD151 and in TEX (bold red), but not of CD63 and CD81 indicating that sExo/TEX are with an unexpectedly high preference derived from TEM-located CD151 and Tspan8 (**Table 1**). Only uptake of dbko-sExo/TEX was slightly

Table 1. Recovery of tetraspanins in MCA tumors and TEX.

	Tumor cells				TEX			
	CD63	CD81	CD151	Tsp8	CD63	CD81	CD151	Tsp8
wt MCA	30%	30%	30%	15%	35%	30%	59%	**46%**
Tsp8ko MCA	24%	19%	22%	nd	35%	29%	57%	<3%
CD151ko MCA	22%	18%	nd	12%	34%	31%	<3%	**46%**
dbko MCA	23%	18%	nd	nd	36%	29%	<3%	<3%

nd: not detected

affected. The latter suggests that sExo/TEX Tspan8 and CD151 can mutually replace or, alternatively, additional tetraspanins (e.g. CD63 or CD81) could support binding and uptake.

Uptake of sExo may provide a continuous modulation and uptake of TEX may account for reprogramming. In advance of progressing with selected activities of sExo/TEX CD151 and Tspan8 activities, we want to mention a very elegant and informative in depth review of molecular functions of Tspan8 that has recently been published [43]. This review includes transcription of Tspan8 as well as its functional activity as (co)transcription factor (TF) that we did not explore. Finally, we apologize that we will not discuss CD9. CD9 was first described to be involved in sperm-egg fusion [44]. It is expressed on most cells, including leukocytes and is enriched in sExo/TEX [45,46]. However, it is not recovered in TEM. Taking into account only activities of TEM-derived sExo and TEX, we could not detect any measurable impact of CD9.

Section 2. Tspan8 and CD151 and cancer

The potency of our model relied on the criss-cross evaluation of wild-type (wt), Tspan8ko and/or CD151ko cells as well as sExo/TEX in wt, Tspan8ko and/or CD151ko mice. Yet, due to the criss-cross evaluation with 16 samples per assay, our findings largely remained at a descriptive level. Our comment tries to cope with this constraint by focusing on publications elaborating underlying mechanisms.

We will discuss the engagement of Tspan8 and CD151 in

2a. Tumor induction/growth

2b. Tumor cell adhesion and migration

2c. Tumor progression and

2d. the contribution of proteases to tumor invasion

2a. CD151 and tumor induction: Methylcholanthrene (MCA)-induced tumors express all common tumor markers (ALDH1/2, CD133, CD24, S100A4, HSP70, CD184, TGFβ1, CD138, ThbS, TF). Distinct to epithelial cancer induction in CD151ko mice [47], MCA-tumor induction was not affected in Tspan8ko and/or CD151ko mice. The more rapid local growth of a dbko tumor fortifies the suggestion that neither Tsp8 nor CD151 are linked to MCA tumor induction. Nonetheless, CD151 may contribute to oncogenicity in epithelial tumors via the crosstalk with the surrounding that promotes integrin activation initiating either focal adhesion kinase (FAK) or RTK activation and downstream signaling [48–53].

2a1. β4 associated CD151 promotes proliferation and invasion by associating with MET (hepatocyte growth factor (HGF) receptor), where CD151 forms a complex with MET and β4 that promotes β4 phosphorylation and association with growth factor receptor binder 2-associated binder (Grb2-Gab1). This signaling pathway leads to mitogen-activated protein kinase (MAPK) stimulation. A CD151ko abolishes this signaling pathway [50].

2a2. Breast cancer (BC) cells expressing the CD151 complex show enhanced Erb-B2 Receptor Tyrosine Kinase 2 (ErbB2) phosphorylation. The effect was only observed in cells expressing the α3β1-CD151 complex. The authors conclude that α3β1-CD151 promotes ErbB2 signaling [51].

2a3. CD151-deficient tumors exhibited hyperactivation of the Wnt pathway as reflected by a marked upregulation in β-catenin (β-cat) and Cyclin D1, and their target genes. In addition, E-cadherin (E-cadh) displayed a cytosolic distribution and TF Snail was markedly upregulated, implying that CD151 suppresses Proto-oncogene Wnt-1 (Wnt1)-driven oncogenicity [52].

2a4. Resistance to anti-ErbB2 of BC was elaborated that relied on adhesion of ErbB2+ BC cells to basement membrane laminin-5. Knockdown of laminin-binding integrins (α6β4, α3β1) or associated CD151 reversed laminin-5 resistance. A CD151 knockdown (kd) inhibited ErbB2 activation and downstream signaling through AKT (serine/heroine kinase), extracellular signal regulated kinases (Erk1/2) and FAK. The authors conclude that the function of ErbB2 in BC is promoted by integrin-mediated adhesion to laminin-5, with strong support by CD151, leading to signaling through FAK. Consequently, removal or inhibition of any of these components (laminin-5, integrin, CD151, FAK) markedly sensitizes cells to anti-ErbB2 agents [51].

The multiple signaling pathways where FAK is involved are discussed in detail in an elegant review, where it is important to be aware that TEM-located tetraspanin-integrin complexes trigger FAK phosphorylation, which ultimately triggers the downstream binding partners of FAK [53]. The findings are summarized in (**Table 2a)**.

2b. Tspan8 and CD151 and tumor cell adhesion and migration: A linkage of Tspan8 and CD151 to cytoskeleton proteins suggests an impact on cell migration [54] repeatedly demonstrated for Tsp8 and CD151 [55–64]. Some examples are mentioned.

2b1. Tspan8 prompts melanoma cell detachment from their microenvironment and triggers melanoma cell invasiveness. The authors demonstrated that β-catenin (cat) stabilization is a molecular signal subsequent to the onset of Tspan8 expression, and that, in turn, β-cat triggers the direct transcriptional activation of Tsp8 expression, leading to melanoma invasion. β-cat activation systematically correlates with a high Tsp8 expression in melanoma that is characterized by a combined activation of β-cat and mitogen-activated protein kinase (MAPK) signaling. These data suggest that β-cat and Tspan8 are part of a positive feedback loop, which sustains a high Tsp8 expression level and confers invasive properties to melanoma cells [64].

Table 2a. CD151 and tumor induction.

Donor	Target	Impact	Signaling	Ref
CD151-β4	GC line	Prol./invasion	CD151-β4-MET complex→ β4p-Grb2complex→MAP	[50]
CD151-α3β1	BC	Prol./invasion	CD151-α3β1→ErbB2 signaling ↑	[51]
CD151	BC	Survival	CD151ko→Wnt pathway↑/β-cat↑/cyclinD1↑ and targets↑, cytosolic E-cadh Snail↑→CD151: EMT↓	[52]
CD151/α3β1/α3β4	BC	ErbB2 resistance	CD151kd→ErbB2↓, Akt, ERK1/2, FAK,→CD151/α3β1/α3β4: laminin adhesion	[51]

2b2. In colorectal cancer (CRC) cell migration and metastasis is supported by Tspan8. The phenomenon is not observed after silencing E-cadherin (E-cadh) or its associated adapter molecule p120cnt. E-cadh directly interacts with Tspan8, the interaction involving a switch in signaling between the collagen-binding integrins α1β1 and α2β1. Cell motility was severely reduced by antibody-mediated disruption of Tspan8 only when p120ctn was silenced. Thus, Tspan8 modifies cancer cell motility via an adhesion signaling network [63].

2b3. CD151 also contributes to cell migration via a linkage of the membrane type 1-matrixmetalloprotease (MT1-MMP) to ezrin/radixin/moesin (ERM), which is only observed in CD151 expressing TEM. The linkage of ERM and MT1-MMP depends on the cytoskeleton, which is regulated through its incorporation into TEM. The authors identified a juxtamembrane positively charged cluster responsible for the interaction of MT1-MMP with ERM, cytoskeleton connectors in BC cells. Linkage to ERM regulates MT1-MMP subcellular distribution and internalization. MT1-MMP association to ERM and insertion into TEMs are independent phenomena, so that mutation of the ERM-binding motif in the cytoplasmic region of MT1-MMP does not preclude its association with CD151, but impairs the accumulation and association of CD151/MT1-MMP complexes with actin-rich structures. At the plasma membrane MT1-MMP autoprocessing is severely dependent on the ERM association, which dominates the enzyme's collagenolytic activity [58].

2b4. CD151 and tightly associated α3β1 are localized at the basolateral surface of epithelial cell. Overexpression of CD151 accelerates intercellular adhesion, whereas treatment of cells with anti-CD151 perturbed the integrity of cortical actin filaments and cell polarity. E-cadh puncta formation, indicative of filopodia-based adhesion zipper formation, as well as E-cadh anchorage to the detergent-insoluble cytoskeleton matrix was enhanced in CD151-overexpressing (CD151oe) cells. GTP-bound Rho GTPase subfamily of small G-proteins of the Ras superfamily-cell division control protein2 (cdc42) and rac (subfamily of the Rho family of GTPases) were also elevated in CD151oe. This suggested an involvement of CD151 in E-cadh-mediated actin cytoskeleton reorganization. Consistent with this possibility, engagement of CD151 by fixed CD151 monoclonal antibody (mAb) induced prominent Cdc42-dependent filopodia extension, which along with E-cadh puncta formation, was strongly inhibited by a protein kinase C (PKC) inhibitor. The authors suggest that CD151 is involved in epithelial cell-cell adhesion by modulating PKC- and Cdc42-dependent actin cytoskeleton reorganization [59].

2b5. CD151 associates with laminin-binding α3β1/α6β1 in epithelial cells and regulates adhesion-dependent signaling events. CD151 is involved in recruiting rat sarcoma virus small GTPase (ras), rac1, and cdc42 to the cell membrane region, leading to the formation of CD151/α3β1/α6β1-GTPases complexes. Furthermore, cell adhesion to laminin enhances the CD151-β1 association, thereby increasing complex formation between β1 and the small GTPases. Adhesion receptor complex-associated small GTPases were activated by CD151-β1 complex-stimulating adhesion events. Additionally, FAK and proto-oncogene protein tyrosine kinase (src) appeared to participate in this adhesion-dependent activation of small GTPases. The phenomenon was not observed in CD151kd cells. Small GTPases activated by engagement of CD151-β1 complexes contributed to CD151-induced cell motility and matrixmetalloproteinase (MMP)9 expression in human melanoma cells. Importantly, among tetraspanins that associate with β1, only CD151 exhibited the ability to facilitate complex formation between β1 and small GTPases and to stimulate β1-dependent activation of small GTPases. These results suggest that CD151 links α3β1/α6β1 to ras, rac1, and cdc42 by promoting the formation of multimolecular complexes in the membrane, which leads to the up-regulation of adhesion-dependent small GTPase activation. Thus, the authors elaborated a new mechanism by which integrins transducer signals to downstream small GTPases become involved in cell-cell and cell-extracellular matrix (ECM) adhesion [60].

Finally, we should at least mention an alternative mechanism accounting particularly for ERM protein activation that proceeds via tetraspanin-associated CD44 [65–67].

Taken together, it is notable that Tspan8 and CD151 frequently are involved in organization of the cytoskeleton and cytoskeleton related signal transduction that mostly proceeds via activation of ERM, initiated by Tsp8 or CD151 associated molecules. Distinct activities of Tsp8 and CD151 mostly rely on associated adhesion molecules that differ for Tsp8 and CD151 (**Table 2b**).

2c. Tspan8 and CD151 and tumor progression: Tumor cell dissemination was evaluated by *ex vivo* outgrowth of tumor cells in blood, dispersed lymph nodes (LN), bone marrow (BM), lung and liver. Disseminated tumor cells were recovered in 26 wt mice bearing a wt tumor, in 17 wt mice bearing a Tspan8ko tumor, in 15 wt mice bearing a CD151ko tumor and in 12 wt mice bearing a dbko tumor. One way towards tumor cell dissemination would be acquisition of anchorage independent growth and epithelial-mesemchymal transition (EMT) [68]. However, the impact of Tspan8 and CD151 on anchorage independent growth as well as on EMT were minor and did not reach a significant level. Metastasizing tumor cells are also characterized by apoptosis resistance [69,70], where TEX are supposed to be of major importance [71–74]. However, apoptotic

Table 2b. Tumor cell adhesion and migration.

Donor	Target	Impact	Signaling	Ref
Tsp8	Melanoma	Invasion	β-cat triggers Tsp8 expression and vice versa→high Tsp8: invasiveness	[64]
Tsp8	CRC	Motility	Tsp8-E-cadh/p120-cat→switch between α1β1 and α2β1→ p120-cat-integrin association disrupted	[63]
CD151	BC	Migration	CD151-ERM-MT1-MMP juxtamembrane cluster→association with cytoskeleton, mutation of MT1-MMP-ERM binding motifà reduced proteolytic activity	[58]
CD151-α3β1	Epithelium	Motility	CD151oe: E-cadh anchorage to ECM↑ and to cortical actin filaments↑, Cdc42↑ Rac↑	[59]
CD151-α3β1/α6β1		Adhesion	Laminin adhesion CD151-β1→complex with Ras↑, Rac1↑, Cdc42↑→ECM adhesion	[60]

receptor expression was unaltered and a minor reduction of BclXl and pBAD in Tsp8ko and CD151ko cells and TEX did not affect apoptosis resistance. Instead, tumor cell dissemination also relies on a crosstalk with the surrounding [75–89] and adhesion molecules can contribute to tumor cell migration [75,90–93]. The adhesion molecules α3, the transmembrane glycoproteins CD105 (endoglin) and CD62L (L-selectin) are reduced in Tspan8ko and dbko cells and TEX, and CD56 and CD106 are reduced in CD151ko and dbko cells and TEX. α6 is reduced in all 3 ko cells and TEX [32]. Thus, we searched for publications that elaborated mechanisms of tetraspanin-adhesion molecule supported tumor cell dissemination.

Only few reports evaluate the molecular mechanism whereby Tspan8 promotes tumor progression. We will mention 2 reports evaluating the molecular mechanism.

2c1. Melanoma is well known for its propensity for lethal metastasis and resistance to most current therapies. Tumor progression and drug resistance depend to a large extent on the interplay between tumor cells and the surrounding matrix. The authors identified Tspan8 that is not expressed in healthy skin as a critical mediator of melanoma invasion. Tspan8 may influence cell-matrix anchorage and regulate downstream molecular pathways leading to aggressive behavior. The authors uncovered that Tspan8-mediated melanoma invasion resulted from defects in cell-matrix anchorage by interfering with Tspan8-β1 clustering without affecting global expression levels. The defect in clustering was associated with impaired phosphorylation of integrin-linked kinase (ILK) and its downstream target AKT-S473, but not FAK. A blockade of AKT or ILK activity strongly affected cell-matrix adhesion. Moreover, expression of a dominant-negative form of ILK reduced β1 integrin clustering and cell-matrix adhesion. The authors confirmed this tumor-promoting effect by a mutually exclusive expression pattern between Tspan8 and phosphorylated ILK *in vivo*. The authors conclude that Tspan8 promotes melanoma progression by negatively regulating ILK activity. Targeting Tspan8 may prevent tumor dissemination by switching from low to firm-adhesion [94].

2c2. Tspan8 is implicated in supporting gastric cancer (GC) progression. The authors found significantly elevated Tspan8 expression in GC tissues that correlated with poor differentiation, invasion depth, LN metastasis, clinical stage and poor prognosis. Functionally, a Tspan8kd significantly reduced, while Tspan8oe assisted GC cell migration and invasion. *In vivo*, a Tspan8kd suppressed lung metastasis in nude mice. In concern about the underlying mechanisms, the authors observed that Tspan8 regulates the epidermal growth factor receptor (EGFR) expression in GC cells by accelerating phosphorylation of EGFR and AKT. The authors suggest that Tspan8 might be a promising therapeutic target [95].

There is no evidence for CD151 accounting for tumor induction. Instead, there are numerous reports on its engagement in tumor progression. Only few of those describing the underlying mechanism will be mentioned.

2c3. Several studies report on a correlation between CD151 expression and BC metastasis. One study describes that CD151 expression was high in BC and expression levels correlated with tumor stage, LN and distant metastases. A CD151kd reduced cell proliferation, invasion and migration. Western blot (WB) demonstrated that the CD151kd was accompanied by a significant decrease of TGF-β1, p-Smad2, p-Smad3, N-cadh, vimentin (vim) and the metalloproteinase (MMP)9, where it is known that Smad is the only known intracellular TGFβ1 receptor (TGFβ1R) substrate. In view of this background information, the authors conclude that CD151 promotes BC metastasis by upregulation of the TGF-β1/Smad pathway. They did not comment on the downregulation of MMP9 [96].

2c4. In another study on the impact of CD151 and associated integrins on BC growth and metastasis, the authors had noted that a CD151ko specifically attenuates TGFβ1-induced scattering and proliferation of BC cells in three-dimensional matrigel. CD151-dependent cell scattering requires its association with either α3β1 or α6β1. CD151 regulates the compartmentalization of TGFβRI and specifically controls the TGFβ1-induced activation of p38 mitogen-activated protein kinases (p38). In contrast, signaling leading to activation of small mother against decapentaplegic (Smad)2/3, AKT, and extracellular signal regulated kinases (Erk)1/2 proteins were unimpaired in CD151ko cells. Attenuation of TGFβ1-induced responses correlated with reduced retention in the lung vascular bed and accordingly with lung metastasis. The authors conclude that they unraveled the contribution of CD151-α3β1 or -α6β1 complexes to TGFβRI signaling, where regulation of signaling plays an important role in BC metastasis [97]. We want to add that the authors had published a related study several years in advance of the above cited publication. Unfortunately, there are some discrepancies on the involved TGFβRI-related signal transduction. Thus, the precise signaling pathway remains to be explored.

2c5. To unravel whether CD151 contributes to BC progression, the authors used CD151ko mice expressing the mouse mammary tumor virus (MMTV)-Erb-B2 receptor tyrosine kinase 2 (ErbB2) transgene. CD151 strongly supports ErbB2+ BC growth and metastasis. In the CD151ko mice tumor onset was delayed and survival of mammary epithelial cells was impaired. Both primary tumors and metastatic nodules showed smooth, regular borders, consistent with a less invasive phenotype. Consistent with decreased metastasis, the MMTV-ErbB2 transgene expressing tumor cells showed substantial decreases in three-dimensional colony formation, epidermal growth factor (EGF)-stimulated tumor cell motility, invasion, and transendothelial migration. CD151-dependent functions were largely mediated through CD151-associated α6β4 and the association with ErbB2. The absence of CD151 was accompanied by impaired activation of FAK- and MAPK-dependent pathways, where CD151 ablation substantially prevented PKC- and EGFR/ERK-dependent α6β4 phosphorylation, which affects epithelial cell polarity and intermediate filament cytoskeleton connections. Thus, there is strong evidence that CD151 collaborates particularly with α6β4 and ErbB2 to regulate multiple signaling pathways, driving mammary tumor onset, survival, and metastasis that remains to be elaborated in detail. Nonetheless, it is obvious that CD151 is a useful therapeutic target in malignant ErbB2+ BC [98].

2c6. Again, in BC, a study investigated the involvement of CD151, hepatocyte growth factor (HGF) receptor (MET), and α3β1/α6β1 in the cellular morphogenesis of human BC cells, where CD151 forms a functional complex with MET and α3β1 and α6β1. A kd of CD151, α3, or α6 abolished branching morphogenesis. Decreased MET expression in these cells led to the formation of rudimentary networks. Furthermore, HGF promotes cellular morphogenesis by accelerating network reorganization.

Immunoprecipitation revealed a direct association of CD151 with MET. The involvement of CD151 and α3/α6 in HGF-dependent signaling was confirmed by the decreased AKT phosphorylation in cells lacking CD151, α3 or α6. The authors conclude that regulation of CD151 expression contributes to changes in HGF/MET signaling and thereby modulates the phenotypic characteristics of cancer cells [99]. The same group confirmed these findings for salivary gland cancer (SVG) cells [100].

2c7. In this study, the authors aimed to examine the role of the CD151-integrin complex in lung adenocarcinoma (LC) metastasis and the underlying mechanisms. They transfected transfect A549 LC cells with a CD151 mutant. *In vitro*, the CD151-AAA (CD151 $QRD^{194-196}$ to CD151 $AAA^{194-196}$) mutant abrogated migration and invasion. The CD151-AAA delivery failed to activate FAK and p130Cas signaling pathways. As CD151 was associated with α3, the authors suggest that the failure of CD151-AAA to associate with α3 accounts for the failure to activate FAK and p130 crk-associated substrate (p130Cas) signaling pathways and that the disruption of the CD151-α3 complex may impair the metastasis-promoting effects and signaling events induced by CD151 in LC. The authors conclude that the CD151-α3 complex promotes LC progression via activation of p130Cas signaling pathways [101]. We want to mention that further precision of the affected pathway is required, as p130Cas is involved in a large array of signaling pathways.

2c8. In another study on LC, highly expressed CD151 was kd. The CD151kd was accompanied by inhibition of proliferation, migration, invasion and colony formation and an enhancement of apoptosis. Furthermore, the expression of tumor development-related proteins, including FAK, PI3K-AKT, Mitogen-activated protein kinase kinase (MEK)-ERK1/2 MAPK as well as the expression of MMP9 and vascular endothelial growth factor (VEGF) were restrained. The study confirms the essential role of CD151 in LC progression. The authors did not dissect the impact of downregulation FAK, PI3K-AKT and MEK-ERK1/2 on metastasis associated molecules as e.g. MMP9 and VEGF, but stress that a blockade of CD151 offers a new possibility for gene therapy to inhibit downstream signaling [102].

2c9. The importance of CD151 in tumor progression was also attacked in another study that used CD151ko mice expressing the MMTV-ErbB2 transgene. CD151 strongly supports ErbB2+ BC initiation and metastasis. In the absence of CD151 survival of mammary epithelial cells was reduced and the FAK- and MAPK-dependent pathways were impaired. Consistent with a less malignant phenotype and decreased metastasis, CD151-targeted mammary epithelial cancer line MCF-10A/ErbB2 cells showed substantial decreases in three-dimensional colony formation, EGF-stimulated tumor cell motility, invasion, and transendothelial migration. These CD151-dependent functions were largely mediated through α6β4. Moreover, CD151 ablation substantially prevented PKC and EGFR/ERK-dependent α6β4 phosphorylation, consistent with retention of epithelial cell polarity and intermediate filament cytoskeleton connections, which helps to explain diminished metastasis. In conclusion, the authors provide strong evidence that CD151 collaborates with laminin binding integrins, particularly α6β4, and ErbB2 and EGFR to regulate multiple signaling pathways, thereby driving BC onset, survival, and metastasis. The authors suggest that CD151 is a useful therapeutic target in malignant ErbB2+ BC [98].

2c10. The authors evaluated the role of α3β1 in ErbB2-dependent proliferation of BC cells in a laminin-rich ECM. Depletion of α3β1 suppressed growth and restored cell polarity. The phenotype of α3β1-depleted cells was reproduced in CD151kd cells and mirrored that of the cells treated with ErbB2 antagonist Herceptin. Cells expressing the α3β1-CD151 complex have higher state ErbB2 phosphorylation and dimerization than α3β1-/CD151-depleted cells. Furthermore, Herceptin-dependent dephosphorylation of ErbB2 was only observed in α3β1-CD151-expressing cells. The level of active RhoA was increased in α3β1- and CD151-depleted cells by Rho controlling ErbB2 dimerization. Taken together, the α3β1-CD151 complex has a critical regulatory role in ErbB2-dependent signaling [59].

We had noted that in MCA tumors CD56 and CD106 selectively coimmunoprecipitated with CD151. As CD56 was recently shown to be involved in the progression of various tumors, it became of interest, whether this association may have an impact on cancer progression and how it affects signal transduction.

2c11. In this study the authors evaluated how cancer associated fibroblasts (CAF) promote cancer progression, which they evaluated using CAF from LC and compared them to fibroblasts (FB) of healthy lungs. Conditioned medium (CM) of CAFs increased proliferation, migration and invasion of LC. CD106 was upregulated and blocking CD106 attenuated the proliferation and invasion of cancer cells. CD106 secreted from CAF activated AKT and MAPK signaling via α4β1. Bioinformatics analysis indicated a positive correlation on the CAF marker alpha-smooth muscle actin (α-SMA) and CD106 expression. The authors concluded that CD106 enhances LC growth and invasion by activating AKT and MAPK signaling [103].

2c12. The authors evaluated the impact of CD56 on melanoma progression. A CD56kd melanoma line affected melanoma proliferation, apoptosis, autophagy, migration, and EMT. Furthermore, the organization of actin was impaired and phosphorylation of cofilin, an actin-cleaving protein was reduced. Transfection with dephosphorylated cofilin exerted similar effects to the CD56kd. Research on the underlying molecular mechanism showed that a CD56kd suppressed activation of the Src/AKT/mammalian target of rapamycin (mTOR) pathway, where the phosphorylation level of cofilin decreased following inhibition of the proto-oncogene protein tyrosine kinase src/AKT/mTOR pathway. The authors conclude that the impact of CD56 on melanoma progression relies on src/AKT/mTOR/cofilin pathway-mediated dynamics of the actin cytoskeleton [104].

2c13. Authors evaluated the impact of CD56 on melanoma growth. A CD56kd inhibited proliferation of B16F10 melanoma cells and colony formation on soft agar and retarded melanoma growth. Change of CD56 expression regulated the activity of Wnt1 signaling molecule β-cat. The signaling cascade included the fibroblast growth factor receptor (FGFR) and glycogen synthase kinase-3β (GSK-3β), but was independent of Wnt receptors, MAPK-ERK and PI3K/AKT. In line with this, CD56 forms a functional complex with β-cat, FGFR, and GSK-3β. The findings suggest that the intracellular domain of CD56 is required for facilitating β-cat signaling. The authors suggest CD56 serving as a new therapeutic target for melanoma treatment [105].

Another group of proteins, GPCR, play a crucial role in cellular signaling including cancer initiation and progression.

GPCR consists of an extracellular N terminus, seven alpha-helical transmembrane domains connected by three intracellular loops and three extracellular loops and an intracellular C terminus. GPCR can translate extracellular stimuli into an intracellular response, through heterotrimeric G proteins consisting of α, β and γ subunits that interact with other proteins and activate a diverse array of downstream signaling pathways. Recently, GPCR have received particular attention in drug discovery. In fact, they are targeted by nearly 34% of all approved drugs [106]. We recommend clinicians that are not already familiar with GPCR to read some reviews on this most prominent drug targets [107]. Here we mention only 3 examples.

2c14. Expression of the GPR19 is elevated in BC and is associated with a CD151-integrin complex. Adropin is an endogenous ligand for GPR19 and activates GPCR19. This, in turn promotes activation of the MAPK/ERK1/2 pathway, essential for upregulation of E-cadh. Thus, overexpression of GPR19 drives mesenchymal-like BC to adopt an epithelial-like phenotype. The recapitulation of epithelial characteristics at secondary tumor sites is an essential step in colonization, where GPR19 plays a decisive role in metastasis by promoting the mesenchymal-epithelial transition through activation of ERK/MAPK pathway via a CD151-integrin-GPCR19 complex that potentiates ligand-induced activation of EGFR and ErbB2 and facilitates receptor homo- and hetero-dimerization [108].

2c15. CXCR4, the receptor of stromal cell-derived factor 1 (SDF-1) is important in tumor progression. The authors evaluated the impact of CXCR4 on CRC progression and unraveled the underlying mechanism. Expression of CXCR4 and associated β-cat were highly increased in CRC compared to normal colon. In addition, expression of E-cadh was low, N-cadh and vim expression was high, suggesting a cross talk between the SDF-1/CXCR4 axis and Wnt/β-cat signaling pathway in CRC. In vitro, SDF-1-induced CXCR4-positive CRC invasion was accompanied by activation of the Wnt/β-cat signaling pathway and was inhibited by the Wnt signaling pathway inhibitor Dickkopf-1. In conclusion, CXCR4-promoted CRC progression is regulated by the Wnt/β-cat signaling pathway. The authors suggest targeting the SDF-1/CXCR4 axis should be considered for clinical applications to suppress CRC progression [109]. We want to add that activation of the Wnt/β-cat signaling pathway by SDF-1/CXCR4 was confirmed in numerous studies.

2c16. Cell-in-cell invasion is an integrin-independent process observed in carcinoma exposed to conditions of low adhesion. Active cell invasion depends on RhoA and actin. Searching for specific cell surface receptors that trigger invasion in a signal-dependent fashion revealed the GPCR LPA receptor 2 (LPAR2) is a signal transducer specifically required for active invasion during entosis. G12/13 (alpha subunits of heterotrimeric G proteins) links GPCR to guanine nucleotide exchange factors (Gab2) and Rho guanine nucleotide exchange factor 11 (PDZ-RhoGEF) required for entotic invasion, which is driven by blebbing and a uropod-like actin structure at the rear of the invading cell. Finally, there is evidence for an involvement of Diaphanous-related formin-1 (Dia1) for entosis downstream of LPAR2. Thus, this GPCR pathway regulates actin dynamics during cell-in-cell invasion [110].

Other studies were predominantly concerned about the engagement of integrins in tumor progression.

2c17. As the precise role of integrins in EGFR activation in LC remained elusive, the authors analyzed human lung tissue samples by methods of molecular biology and biochemistry, performed RNA-sequencing-based transcriptomic analysis, as well as high-resolution mass spectrometric analysis. They identified a novel LC gene expression signature consisting of 93 transcripts that were induced by β2. They also uncovered β2 inducing LC stem cell markers and activating RAS/MAPK/ERK signaling in control human lung tissue [111].

2c18. Another study was concerned about the impact of α3 on cervical cancer (CC) progression. α3 is highly expressed in CC and predicts poor survival. By α3 downregulation the authors could demonstrate that α3 recruits a c-Src/ERK1/2 cascade, leading to phosphorylation of FAK that endows CC cells with high migratory and invasive ability, where α3 is involved in c-Src and ERK1/2 activation and MMP9 expression. The authors conclude that shedding light on the mechanism, whereby α3 is involved in CC metastasis provides a new prognostic biomarker and a therapeutic target [112].

2c19. α3 is strongly upregulated in papillary thyroid carcinoma (PTC) and linked with LN metastases. α3 associates with MET, which promotes phosphorylation of MET at Tyr1234/1235, activation of ERK and the PI3K/AKT signaling pathways. Thus, α3 promotes PTC cell proliferation and migration by cooperating with MET to activate MET-ERK and MET-PI3K-AKT signaling. Targeting the α3-MET cooperation may serve as a potential therapeutic target [113].

Finally, we want to mention at least 2 publications describing that Tspan8 is involved in the maintenance of cancer stem cells (CSC).

2c20. One study describes that Tspan8 is upregulated in breast CSC. Tspan8 promotes expression of the stemness TF NANOG, octamer-binding TF 4 (OCT4), and aldehyde dehydrogenase 1 family, member A1 (ALDHA1), and correlates with therapeutic resistance. Mechanistically, Tspan8 interacts with Protein patched homolog 1 (PTCH1) and inhibits the degradation of the sonic hedgehog (SHH)/PTCH1 complex through recruitment of the deubiquitinating enzyme ataxin 3 (ATXN3). This results in the translocation of Sequential minimal optimization (SMO) to cilia downstream gene expression, resistance of CSCs to chemotherapeutic agents, and enhanced tumor formation in mice. Accordingly, expression levels of Tsp8, PTCH1, SHH, and ATXN3 are positively correlated in human BC, and high Tspan8 and ATXN3 expression levels correlate with poor prognosis. The findings reveal a molecular basis of Tspan8-enhanced SHH signaling and highlight a role for Tspan8 in promoting cancer stemness [114].

2c21. Another study reports that CRC stemness also is driven by Tspan8. A Tspan8kd reduces the expression of stemness genes and sphere forming capacity in CRC. Tspan8 directly interacts with ß-cat and enhances its protein expression. Conversely, ß-cat directly binds to the Tspan8 promoter and enhanced Tspan8 transcription. Persisting high Tspan8 expression via this feedback loop supports CRC stemness maintenance [115].

In brief, the impact of Tspan8 and CD151 on tumor progression can depend solely on the Tspan8 expression level or CD151 associations with integrins, CD56, or GPCR. We want to stress and apologize that this is a rather arbitrary selection. The data are summarized in **Table 2c**.

Table 2c. Tsp8, CD151 and other proteins contributing to tumor progression.

Model	Target	Impact	Signaling	Ref
Melan., *in vivo*	Tsp8	Cell-matrix anchorage↓	Tsp8-β1 clustering↓: ILKp↓/ AKTp↓ /mutual exclusive Tsp8 vs ILK	[94]
GC, *in vivo*	Tsp8	Metastasis	Tsp8 regulates EGFRp /AKTp	[95]
BC	CD151	Metastasis	TGF-β1 /Smad↑↑, vim↑	[96]
BC	CD151	Lung metast./scattering	CD151-α3β1/-α6β1: TGF-β1-induced p38↑	[97]
BC	CD151	Motility	CD151-α6β4α/ErbB2: epithelial cell polarity/cytoskel↓, PKC- and EGFR/ERK↑	[98]
BC, SVG	CD151	Scattering	CD151-α3β1 and -α6β1/MET complex: branching↓	[99,100]
LC	CD151-AAA	Metastasis	CD151-α3 disrupted by CD151-AAA, CD151: FAKp↑ p130Cas↑, p130Cas signaling promotes LC progression	[101]
LC	CD151	Metastasis	FAK↑, PI3K-AKT↑, MEK-ERK1/2 MAPK↑, MMP9↑, VEGF↑	[102]
BC	CD151	Metastasis	CD151-α6β4p: EGFR↑ERKp↑→onset, survival, metastasis↑	[98]
BC	CD151	ECM interaction	CD151-α3β1: ErbB2p↑, RhoA↑	[60]
LC CAF	CD106	Proliferation/metast	CD106-α4β1 CAF: AKT↑, MAPK↑	[103]
Melanoma	CD56	Progression	CD56: Src/AKT/mTOR↑, actin-cleaving cofilinp↑, Src/AKT/mTOR/cofilin organize actin cytoskeleton	[104]
B16F10, *in vivo*	CD56	Growth	β-cat/ Wnt1 signaling (FGFR, GSK-3β) CD56 (intracell.domain)/β-cat/	[105]
BC	GPCR	Met.settlement	GPCR19 activated by Adropin (endog. ligand): MAPK/ERK1/2↑→ E-cadh↑, epithelial-like phenotype: promotes metastasis settlement, potentiated by CD151/integrin/GPCR19 complex	[108]
CRC	CXCR4	Progression	Crosstalk SDF-1/CXCR4 axis and Wnt/β-catenin: SDF-1-induced CXCR4+ CRC Wnt/β-cat↑ inhibited by Dickkopf-1	[109]
CRC	LPAR2	Entosis / invasion	Rock↑, STAT3↑ G12/13, cyclin E1, A2, B1↑, Rock↑ independent of STAT3↑ by linkage to Rock to Gab1, involvement of Dia 1	[110]
LC	β2	LC stem cells	LC stem cell marker β2 induces RAS/MAPK/ERK↑	[111]
CC	α3	Progression	α3 recruits c-Src→ERK1/2↑, FAK↑, MMP9↑	[112]
PTC	α3	Metastasis	α3 associates with MET→METp↑, ERKp↑ PI3K/AKT↑	[113]
BC	Tsp8	CSC	Tsp8 promotes stemness genes NANOG, OCT4, ALDHA1, Tsp8/PTCH1 degradation inhibited by ATXN3→translocation of SMO	[114]
CRC	Tsp8	CSC	Tsp8 / ß-cat mutual transcription, high Tsp8 supports stemness	[115]

2d. The contribution of proteases to tumor progression: Matrix degradation, can contribute to tumor cell motility by paving a path for migrating tumor cells, increased protease expression in TEX fitting to their association with tetraspanins [82–86]. Reduced expression of all tested proteases in Tsp8ko cells points towards a preferential association with Tsp8, only TACE and ADAM10 are also reduced in CD151ko cells and TEX. Wt TEX degradation was partly maintained in single Tspan8ko and CD151ko TEX, but not dbko TEX. Several possible pathways were described. Some examples exploring the underlying mechanism are mentioned.

2d1. Tumor vasculature is frequently leaky. One study evaluated the underlying mechanism. Vitronectin (VTN) rapidly and potently activates leukemia inhibitory factor (LIF) and pro-inflammatory interleukin 6 (IL-6) after vascular injury that was accompanied by FAK activation, a FAKkd reducing the expression of LIF and IL-6. VTN effects also were inhibited by blocking αvβ3 and αvβ5 and Arg-Gly-Asp (RGD) and by an uPARkd, uPAR being known to bind VTN through a common target of uPAR and αvβ3 and αvβ5. Thus, αvβ3 and αvβ5-FAK signaling promotes inflammation. The authors suggest that blocking αvβ3 and αvβ5 and uPAR can efficiently hamper tumor vasculature leakage [81].

2d2. uPAR plays an important role in cancer pathogenesis, including breakdown of ECM, invasion, and metastasis. The authors evaluated the role of uPAR in PTC, the most frequent endocrine thyroid malignancy. They noted that an uPARkd greatly reduced migration and invasion. A common mutation in PTC leads to ERK1/2 hyperphosporylation, where inhibition of ERK1/2 phosphorylation was accompanied by a reduction of uPAR. An uPARkd was accompanied by pronounced FAK/PI3K/AKT signaling. Concomitantly, proliferation and cell survival drastically decreased. The authors interpret their findings that uPAR induction is a consequence of ERK1/2 activation and that uPAR is a central component in the pathogenesis of PTC. Targeting uPAR might provide an efficient therapeutic [82].

2d3. Nasopharyngeal carcinoma (NPC) is one of the most common malignancies in southern China and Southeast Asia with a high metastasis rate. To understand the mechanisms underlying NPC progression the authors performed genome-wide expression profiling on 18 NPC vs. 18 noncancerous nasopharyngeal tissues. Together with GeneGo pathway analysis and expression verification in NPC cells and tissues revealed a potential role of uPAR, uPAR being significantly increased in poorly differentiated, highly

metastatic NPC cells compared with lowly metastatic cells. *In vitro* studies demonstrated that uPAR regulates NPC cell growth, colony formation, migration, and invasion and promotes metastases in a xenograft model. Immunoblotting indicated an involvement of the JAK-STAT pathway in uPAR-regulated signaling. Accordingly, uPAR-mediated growth and motility was partially abolished upon treatment with the Janus kinase (JAK)1/2 inhibitor INCB018424. The authors conclude that uPAR is a significant regulator of NPC progression and could serve as a promising therapeutic target [84]. The finding was confirmed by UTI (ulistatin), an urinary trypsin inhibitor that suppressed uPA, uPAR, and JAK/STAT3 signaling accompanied by a significant decrease in metastasis [116].

2d4. In this study, the authors evaluated the impact of ionizing radiation (IR) on uPAR activity in medulloblastoma. IR induced the expression of uPAR and CSC markers, such as Musashi-1 and CD44, and activated WNT-7a-β-cat signaling molecules. Independent of IR, overexpression of uPAR led to increased WNT-7a/β-cat/T cell factor/lymphoid enhancer factor family (TCF/LEF)-mediated transactivation, which promotes cancer stemness. Instead, an uPARkd suppressed WNT-7a/β-cat/TCF/LEF-mediated transactivation similarly to a potent WNT/β-cat inhibitor. Recombinant WNT-7a induced uPAR, indicating the existence of a mutual regulatory relationship between uPAR and WNT/β-cat signaling. Furthermore, uPAR is physically associated with the WNT effector molecule β-cat on the membrane, cytoplasm, and nucleus of IR-treated cells and CSC, where in the nucleus uPAR associates with TF and their specific response elements like activating enhancer-binding protein 2α (AP2a) and mediates β-cat gene transcription. The findings strengthen the importance of uPAR as a potent activator of stemness [83].

2d5. In this study, the authors describe a proteome analysis of the secretome of CRC HT116 in comparison to HT116-MMP2kd. They identified a plausible association between MMP-2 up-regulation and activation of FAK. Based on this and additional comparative and integrative analyses, the authors suggest that the high invasiveness of metastatic CRC results from increased secretion of MMP2-tetraspanin-integrin complexes that promote FAK signaling [117].

2d6. MMP7 is secreted as a proenzyme (proMMP7) and plays a key role in the degradation of various ECM and non-ECM molecules after activation. The authors performed a library screening of rectal carcinoma (RC) that revealed an association with CD151 confirmed by co-immunoprecipitation. Deletion mutants of proMMP7 and CD151 suggested an interaction between the propeptide of proMMP7 and the large extracellular loop of CD151 that was confirmed by binding of (125)I-labeled proMMP7. *In situ* zymography using a known substrate of MMP7, demonstrated proteinase activity of CD151-associated MMP7. Activity was abolished by MMP7 mAB or CD151 mAB. The data demonstrate that proMMP7 is captured and activated on the cell membranes through interacting with CD151 suggesting that the CD151-MMP7 association contributes to invasion and metastasis [118].

2d7. Osteosarcoma (OS) is a primary bone malignancy with high early metastatic propensity. The authors aimed to find specific protein targets to develop therapeutic strategies against this lethal disease. CD151 being known to facilitate tumor metastasis, the authors used a CD151kd that, indeed, inhibited OS migration, invasion and metastasis. The authors found that CD151 modulates MMP9 expression through GSK-3β/β-cat signaling. They concluded that CD151 may be a useful antimetastatic target in OS [119].

2d8. Triple-negative breast cancer (TNBC) is characterized by overexpression of CD151 and MMP9, which are independent prognostic factors. Compared with a CD151kd, a double MMP9kd/CD151kd further promoted cell death and inhibited TNBC proliferation, migration, and invasion. Importantly, β-cat and glycogen synthase kinase-3 beta (p-GSK-3β) were significantly downregulated. CD151 and MMP9 jointly blocking GSK-3β/β-cat offers a pathway of therapeutic interference [97].

2d9. Disintegrin-metalloproteinase (ADAM)15 plays important roles in various cancers. The authors evaluated its role in LC. ADAM15oe promotes proliferation, migration and invasion, while an ADAM15kd inhibits these activities. EGFR and ErbB2 phosphorylation were upregulated in ADAM15oe and downregulated in ADAM15kd cells. This accounted for a reduction of p-FAK, p-AKT, p-ERK, cyclin D1 and MMP9, while the total FAK, AKT and ERK levels remained unchanged. ADAM15 can bind to αVβ3. An αVβ3kd reduced the increase in FAK, AKT and ERK phosphorylation; however, it did not affect the phosphorylation of EGFR/Her2. To approach this question the authors evaluated the impact of ADAM15-CD151 coexpression. A CD151kd was accompanied by impaired EGFR-FAK signaling, which relied on the CD151 association. A CD151kd was accompanied by decreased ADAM15 and α3 and α6 expression. Concomitantly, EGFR and FAK signaling were impaired. Thus, signal transduction varies considerably by the CD151-associated integrins. Searching for a target that inhibits ADAM15 expression, they identified miR-204-5p as a direct target. The authors conclude that they unraveled the pathways whereby ADAM15 promotes metastasis in LC and uncovered that miR-204-5p efficiently hampers ADAM15 expression [120].

Taken together, protease upregulation/activation relies, at least in part, on tetraspanins, tetraspanin associated proteases and tetraspanin-associated integrins. Though only selected samples are presented, these don't provide evidence for a unifying concept. Nonetheless, all cited publications point towards therapeutic interference in the given context. Data are summarized in **Table 2d**.

Section 3. CD151 and Tspan8 and the crosstalk between tumor and bone marrow

Impaired settlement of Tspan8ko and CD151ko tumor cells in the autochthonous BM, partly restored in the wt host, suggested a contribution of sExo/TEX Tspan8 and CD151 to tumor cell settlement in the BM. The BM is a preferred metastatic organ [121,122], where an impact of the tumor cells and TEX on metastasis in the BM including hematopoiesis was repeatedly described [123–128].

Tspan8 and CD151 are expressed at a low level in the BM on early hematopoietic progenitors, proto-oncogene c-KIT (KIT)+ cells, KIT+/CD34+ cells being reduced in Tspan8ko and CD151ko BMC, where Tspan8 and CD151 associate with a small subpopulation of hematopoietic progenitor cells. In addition, Tsp8 and CD151 are associated with CXCR-motif-chemokine receptors (CXCR)3 and 4 and with EpHA4. Wt TEX affected expression of CXCR3/CXCR4, KIT and Forkhead box O3 (FOXO3), the central TF in hematopoiesis [129]. We noted that anti-KIT, anti-CXCR3 and anti-CXCR4 precipitated Tspan8 only in wt and CD151ko

Table 2d. Inflammation and proteases and tumor progression.

Start	Target	Impact	Association and signaling	Ref
VTN	Tumor vessels	Vessel leakiness	VTN: →FAK→LIF+IL6↑ via αvβ3 and -αvβ5 → inflammation	[81]
uPAR	PTC line	Migration/invasion↑↑	PTC mut→ERK1/2↑, uPARkd→FAK/PI3K/AKT, β-galactosidase↓↓	[82]
uPAR	NPC	Migration/invasion/EMT↑↑	Jak1/Jak2↑↑	[84,116]
uPAR	Medulloblastoma	Stemness↑↑	uPARoe <-> WNT-7a/β-cat/TCF/LEF↑, uPAR/ β-cat (membrane, cytoplasm, nucleus, in the nucleus together with AP2a→TF: β-cat↑	[83]
MMP2	CRC	Motility/adhesion↑	MMP2 upregulation via FAK/scr↑ and ERK↑	[117]
MMP7	RC	Invasion/metastasis↑	CD151 captures proMMP7→MMP7 activation→ matrix degradation	[118]
MMP9	OS	Migration/invasion/metastasis	CD151kd: GSK-3β/β-cat↓↓	[119]
MMP9	BC	Migration/invasion↑↑	CD151kd/MMP9kd jointly block GSK-3β/β-cat	[97]
ADAM15	LC	Proliferation/metastasis	ADAM15-αVβ3→ EGFR↑, FAK↑, CD151-α3/α6→ EGFR↑, FAK↑	[120]

BMC, and CD151 only in wt and Tspan8ko BMC. Our review being particularly concerned about signaling, we searched for literature evaluating KIT, CXCR4 and CXCR3 signal transduction in hematopoietic stem cells (HSC).

3.1 KIT: The RTK KIT is involved besides others in hematopoiesis:

3.1.1 First to note, this accounts already for very early stages of embryogenesis [130–133]. By single cell expression studies of ko-mice, the authors uncovered that KIT is required for transient definitive hematopoiesis in the fetal liver [130]. In another study, the authors used anti-KIT to investigate the expression and function of KIT in murine fetal hemopoietic progenitor cells. They showed that hemopoiesis in the yolk sac and fetal liver started from cells that express KIT, the KIT+ cell population being enriched in hemopoietic progenitors. By using a KIT blocking mAB *in vivo*, they showed that by application after 12.5 days (d) of gestation liver hematopoiesis was severely impaired [131].

3.1.2 Another study evaluated KIT and SCF expression during human embryogenesis in the yolk sac, the paraaortic splanchnopleura/aorta, gonad, mesonephros region (AGM). Precursors of hemopoietic cells are found in this region before circulation is established between the yolk sac and the embryo proper. These observations suggest that the biological function of KIT plays an important role in early stages of human haemopoiesis [132].

3.1.3 Stem cell factor (SCF) also was shown to be critical in the survival and development of stem cells, mutations in KIT being associated with a variety of human diseases. Interaction of SCF with KIT rapidly induces receptor dimerization and autophosphorylation. Downstream of KIT, multiple signal transduction components are activated, including PI3K, Src family members, the JAK/STAT pathway and the ras/raf/MAP cascade [133].

3.1.4 Trying to evaluate the impact of KIT in HSC regulation revealed that the cytoplasmic Src homology region 2 domain-containing phosphatase-2 (shp2), promotes KIT gene expression, constituting a KIT-shp2 signaling axis. Ablation of shp2 resulted in severe cytopenia in BM, spleen, and peripheral blood of mice. Shp2 removal suppressed the functional pool of HSC and shp2-deficient HSC failed to reconstitute lethally irradiated recipients. Shp2 regulates coordinately multiple signals involving up-regulation of KIT via the TF GATA-binding factor 2 (Gata2) [134].

3.1.5 Another study evaluated HSC maintenance in response to stress using a mouse model of acute thrombocytopenia to investigate the cross talk between HSC and niche cells. This process requires membrane-localized SCF, which is regulated, in turn, by VEGFA and platelet-derived growth factor-BB (PDGF-BB). HSC are subsequently activated by a dual-RTK-dependent signaling event, SCF/KIT and VEGFR (VEGF receptor) 2. The finding unraveled the important role of 3 RTKs and their ligands in orchestrating HSC maintenance [135].

3.1.6 The authors of another publication refer to previous reports on a combination of drugs inhibiting GSK3β, p38MAPK, mTOR and histone deacetylase (HDAC) to suppress HSC. They aimed to determine the effect of HDAC, mTOR, GSK-3β and p38MAPK inhibitor combinations on the efficient expansion of HSC, specifically how inhibitors of GSK3β signaling, in combination with inhibitors of P38MAPK and mTOR signaling or HDAC inhibitors could affect HSC expansion. The results indicated that p38MAPK and/or GSK3β inhibitors increased the number of HSC, indicating that the suppression of p38MAPK and/or GSK3β modulate HSC differentiation and self-renewal [136].

3.1.7 Balance between the HSC duality to either possess self-renewal capacity or differentiate into multipotency progenitors is crucial for maintaining homeostasis of the HSC compartment. KIT, activated by its ligand SCF, originating from niche cells, is essential to retain the HSC self-renewal activity. The authors show that AT-rich interaction domain 4B (ARID4B) interferes with KIT/SCF signaling promoting HSC differentiation. ARID4Bko in mouse hematopoietic cells block fetal HSC differentiation, preventing hematopoiesis. Mechanistically, ARID4Bko HSC self-express SCF and overexpress KIT. Downstream of SCF/KIT signaling, inhibition of src family kinases rescues the HSC differentiation defect elicited by the ARID4Bko. In summary, the intrinsic ARID4B-SCF/KIT axis is an HSC program enabling the differentiation state, while KIT/SCF preserves undifferentiated HSPC [137].

3.1.8 FLT3 and KIT being crucial regulators of HSC, the authors investigated the role of space transportation system (STS)1 and 2 on FLT3 and KIT phosphorylation, activity, and function in normal and stress-induced hematopoiesis. STS1/STS2ko mice showed a profound expansion of multipotent progenitor cells. STS1/STS2 are direct phosphatases of FLT3 and KIT. Loss of STS1/STS2 induces hyperphosphorylation of FLT3, enhances AKT signaling, and confers a strong proliferative advantage to HSC [138].

3.1.9 Gain and loss-of-function KIT mutants suggest that small changes in KIT signaling profoundly affect HSC function. The authors demonstrate that even the most rigorously defined HSC can be separated into functionally distinct subsets based on KIT activity. Functional and transcriptome studies show HSC with low levels of surface KIT expression exhibit enhanced self-renewal and long-term reconstitution. Furthermore, KIT(hi) HSC arise from KIT(lo) HSC, where KIT(hi) HSC give rise to lymphomyeloid cells inhibiting the megakaryocytic lineage. Finally, they show that the transition from KIT(lo) to KIT(hi) HSC is negatively regulated by E3 ubiquitin-protein ligase Cbl (c-Cbl). The study demonstrates that HSC exhibiting enhanced self-renewal potential versus differentiation into progenitors can be isolated based on KIT expression levels [139].

3.1.10 GRB2-associated-binding protein (Gab)2 plays major roles in coordinating signaling downstream of hematopoietic cytokine receptors. Gab2 can modulate PI3K and MAP activities and regulate the long-term multilineage competitive repopulating activity of HSC. Gab2 may also act in a linear pathway upstream or downstream of STAT5. The authors aimed to determine whether Gab2 and STAT5 function in hematopoiesis in a redundant or non-redundant manner. They generated Gab2 mutant mice with heterozygous and homozygous deletions of STAT5. The heterozygous STAT5 mutant mice revealed deficiencies in HSC, mirrored in the reduced growth response to early-acting cytokines. Importantly, the steady-state engraftment ability was impaired by loss of Gab2 in heterozygous STAT5 mutants. Fetal liver cells isolated from homozygous STAT5 mutant mice lacking Gab2 showed significant reduction in HSC, reduced HSC survival, and a dramatic loss of HSC self-renewal. The data demonstrate new functions for Gab2 in hematopoiesis in a manner that is non-redundant with STAT5, but revealed synergy between STAT5 and Gab2 in HSC self-renewal [140].

3.1.11 The SCF/KIT pathway being of crucial importance in controlling HSC renewal, the authors aimed to further unravel the intracellular regulation of the SCF/KIT pathway in HSC. They report that the TF Zinc finger protein SNAI2 (Slug) functions as a direct transcriptional repressor of KIT in HSC. Conversely, SCF/KIT signaling positively regulates Slug downstream of MYC and TF Forkhead box protein M1 (FoxM1). Intriguingly, KIT expression is induced by SCF/KIT signaling in Slug-deficient HSC. Thus, the balance between Slug and KIT is critical for maintaining HSC repopulating potential, Slug functioning in a novel negative-feedback regulatory loop in the SCF/KIT signaling in HSC [141].

3.2 CXCR3 and CXCR4 are involved in hematopoiesis. Thus, it became interesting to evaluate the underlying mechanisms. Though there are more than 1000 reports on the engagement of CXCR4 in HSC maintenance, only very few are concerned about CXCR4 signaling in HSC.

3.2.1 One report showed that niche intrinsic expression of CXCR4 critically regulates hematopoietic stem and progenitor cell (HSPC) maintenance during steady state, and promotes early hematopoietic regeneration after myeloablative irradiation. At steady state, chimeric mice with wt HSPC and marrow stroma lacking CXCR4 show decreased HSPC quiescence and reduced repopulation capacity. Mesenchymal stroma cells (MSC) were significantly reduced in the BM of CXCR4ko mice, accompanied by decreased levels of the HSPC supporting factors SDF-1 and SCF. In addition, loss of BM stromal cells was more severe in CXCR4ko mice. Transplantation of wt HSPC into CXCR4ko mice demonstrated reduced HSPC homing and early hematopoietic reconstitution. CXCR4 signaling attenuated irradiation-induced BM stromal cell loss by upregulating of the antiapoptotic protein Survivin via the PI3K pathway. The authors conclude that SDF-1-CXCR4 signaling in the stroma microenvironment plays a crucial role in maintenance of HSPC during homeostasis, and promotes niche regeneration and early hematopoietic reconstitution after transplantation [142].

3.2.2 The authors report that HSC homing and engraftment, critical for HSC transplantation, is facilitated by HDAC5 inhibition that is accompanied by highly upregulated CXCR4, which results in enhanced SDF-1/CXCR4-mediated homing into the BM. HDAC5 inhibition increases acetylated transcription factor p65 (p65) levels in the nucleus, which is important for CXCR4 transcription. Inhibition of NF-κB signaling suppresses HDAC5-mediated CXCR4 upregulation. Furthermore, activation of the NFκB signaling pathway via tumor necrosis factor (TNF)α also results in significantly increased CXCR4 surface expression. These results demonstrate a previously unknown negative epigenetic regulation of HSC homing and engraftment by HDAC5 that promotes CXCR4 upregulation [143].

3.2.3 The authors describe the importance of the association of CXCR4/SDF-1 with tyrosine-protein kinase Lyn (Lyn) on hematopoiesis. CXCR4 stimulates Lyn, which is associated with activation of PI3K and a Src-related kinase. The complex is a target of TIK (Bcr-Abl tyrosine-kinase inhibitor), binding of phosphorylated BCR/ABL to Lyn resulting in the constitutive activation of Lyn and PI3K and is accompanied by a total loss of responsiveness to SDF-1. Inhibition of BCR/ABL with the tyrosine kinase inhibitor STI571 restores Lyn responsiveness to SDF-1 signaling. Thus, BCR/ABL perturbs Lyn function and thereby inhibits CXCR4 activity [144].

3.2.4 Both cell-intrinsic and niche-derived, cell-extrinsic cues drive the specification of HSC in the BM. Patients with WHIM syndrome, a rare congenital immunodeficiency caused by mutations that prevent desensitization of the chemokine receptor CXCR4, have an excess of myeloid cells in BM. The authors investigated the effects of increased CXCR4 signaling on the localization and fate of HSC. Knock-in mice bearing a WHIM syndrome-associated *CXCR4* mutation (*CXCR4*^{1013}) phenocopied myeloid skewing of BMC in patients. Whereas the HSC subtype 4 (HSC4) in wt mice differentiate into lymphoid cells, HSC4 in *CXCR4*^{1013} knock-in mice differentiate into myeloid cells. This myeloid rewiring of HSC4 in *CXCR4*^{1013} knock-in mice was associated with enhanced signaling mediated by mTOR and increased oxidative phosphorylation (OXPHOS). HSC4 also supports formation of arterioles in the BM of knock-in mice, suggesting that the loss of extrinsic cues from the perivascular niche contributes to myeloid skewing. Chronic treatment with the CXCR4 antagonist AMD3100 or the mTOR inhibitor rapamycin restores the lymphoid potential of HSC4 in knock-in mice. Thus, CXCR4 desensitization drives HSC towards lymphoid differentiation, but dampen by mTOR-dependent metabolic changes myeloid differentiation [145].

3.2.5 CXCR3, important in T cells, also contributed to HCS maintenance. The authors established CXCR3ko and C-X-C Motif Chemokine Ligand (Cxcl)10ko mice. CXCR3ko and Cxcl10ko HSC showed reduced self-renewal capacity. To study the effect in the BM microenvironment, the authors implanted donor cells into CXCR3ko or Cxcl10ko recipient mice examining donor-

contributed hematopoiesis. Deficiency of Cxcl10 and CXCR3 leads to decreased BM cellularity, but the proportion of HSC is significantly increased. Accordingly, CXCR3ko HSC demonstrate a reconstitution disadvantage in secondary transplantation, Thus, Cxcl10 and CXCR3 affect HSC maintenance without an impact on the maturation of committed progenitors [146].

3.3 Homing into the BM is facilitated by high CD62P and CD44 expression [147–149]. Unfortunately, our MCA tumor model does not allow us to comment on an impact of Tspan8 or CD151, as expression of CD44 was very high (~85%) with as slight reduction in CD151ko and dbko BMC (~65%). CD62P (~45%) was reduced in Tspan8ko and/or CD151ko BMC (~35%-38%) [32]. Nonetheless, sExo highly express CD62P and CD44 and may *in vivo* contribute to homing into BM. For HSC transplantation pre-transplant coincubation with sExo was described to facilitate their uptake [150].

Briefly, our finding that Tspan8 and CD151 coimmunoprecipitate in HSC with KIT, CXCR3 and CXCR4 implies that Tsp8 and CD151 can potentially become involved in any of the described pathways that positively or negatively interfere with HSC maintenance. From the clinical point of view, as particularly Tspan8 is not expressed on myeloid and lymphoid cell and in the BM is exclusively recovered on HSC, collecting CXCR4+/CXCR3+/KIT+/Tspan8+ cells may significantly increase the take rate of transplants. It also helps avoiding contamination by progenitor cells that may have a negative impact on transplant acceptance. sExo can facilitate transplant immigration by the transfer of CD44 and CD62P that they express at a high level. The data are summarized in **Tables 3**.

Section 4. CD151 and Tspan8 and the crosstalk with endothelial cells

Tumor growth depends on angiogenesis [151–153], where I may recommend an overview of angiogenesis and signaling in angiogenesis [154], links between angiogenesis and Tspan8 or CD151 [30,32,57,155–179] being excessively described. Our interest in angiogenesis was evoked by the notion that a highly Tspan8 expressing tumor induced overshooting angiogenesis that provoked a consumption coagulopathy (coagpath). TEX evoked the same effect *in vivo* and induced *in vitro* EC sprouting accompanied by VEGFR, MMP, and uPAR upregulation. The effect could be blocked *in vivo* and *in vivo* by a Tspan8 mAB. This publication dates 19 years back and was the first to demonstrate that Tspan8 overexpressing cells and TEX are involved in angiogenesis [175]. Meanwhile, many studies have confirmed the impact of Tspan8 and CD151 on angiogenesis including the impact of TEX e.g. [57,159], which stimulate beside others the release of TGF-β, VEGF, platelet derived growth factor (PDGF), and Ang1. We will discuss some publications that elaborate the underlying mechanism.

4.1 The authors compared the impact of platelet (PL)-sExo (PL-sExo) with that of endothelial colony forming cell (ECFC)-sExo on enhancing angiogenesis. The response to PL-sExo was stronger than that induced by ECFC-sExo including EC tube formation and branching, indicating that platelet membrane components contain angiogenesis promoting proteins, which was confirmed by fixed platelets not enhancing tube formation. Subsequent experiments revealed that the promotion depended on platelet-surface glycoproteins, as removal of sialic acid from platelet glycoproteins by neuraminidase abolished the enhancement. Furthermore, PL-sExo, but not ECFC-sExo expressed CD151 that was important for angiogenesis induction as pretreatment of platelets with CD151 mAB attenuated the effect. α6/α6β1 also were involved as observed by mAB treatment. PL-sExo strengthened tube formation via src/PI3K signaling. The authors conclude that PL-Exo enhance angiogenesis via CD151-α6β1 complexes that promote src/PI3K signaling [57].

4.2 The authors evaluated whether CD151 in HUVEC cooperate with MET and which molecules would be involved in this cooperation. They noted an involvement of nitric oxide (NO), VCAM-1, and VEGF. They found that proliferation, migration, and tube formation of HUVEC were assisted by CD151 with activation of MET, FAK, and CDC42, suppressed by a CD151kd. This also accounted for NO, vascular cell adhesion molecule-1 (VCAM1,

Table 3. Early hematopoietic progenitor regulation.

Target	Condition/Goal	Requirement / Signaling	Ref
KIT		Multiple signaling PI3K, Src, JAK/STAT, Ras/Raf/MAP	[131–133]
KIT-Shp2	HSC maintenance	Shp2 multiple signals including Kit↑ via GATA2	[134]
KIT-SCF	Stress	Precondition: SCF, VEGFA, PDGF, recovery dual RTK-dependent (KIT, VEGFR2)	[135]
KIT	Self renewal	Requirement: GSK3β, p38MAPK, mTOR and HDAC	[136]
KIT	Maintaining early HSC	Intrinsic ARID4B-SCF/KIT axis, requires GSK3β, p38MAPK, mTOR and HDAC	[137]
KIT	Hematopoietic recovery	STS1/STS2 prevent FLT3 and c-KIT phosphorylation, loss FLT3↑, AKT↑, HSC proliferation↑↑↑	[138]
KIT(hi)/-(lo)	transplantation	Only KIT(lo) support self renewal	[139]
KIT	Gab2/STAT5	GAB2: engraftment, STAT5 growth, synergize self renewal	[140]
KIT/SCF	Slug	Slug downstream Myc, FoxM1, negative feedback loop	[141]
CXCR4	HSC, homing, niche	CXCR4: antiapoptotic survivin↑↑ via PI3K	[142]
CXCR4	Homing, engraftment	HDAC5↓: p65↑, CXCR4 transcription↑ by NF-κB via TNFα	[143]
CXCR4	CXCR4/SDF-1/Lyn	Target of TIK→Lyn↑ via PI3K, loss of SDF-1 response	[144]
CXCR4 mut.	Myeloid/lymphoid diff.	Myeloid rewiring: mTOR↑, OXPHOS↑	[145]
CXCR3	CXCR3ko, Cxcl10ko	CXCR3ko, Cxcl10ko cellularity↓↓, but HSC↑↑	[146]

CD106), and VEGF. The data suggested that CD151 could promote migration, proliferation, tube formation and angiogenesis of HUVEC via MET signaling [155].

4.3 CD151 being upregulated in HUVEC, the study explored the impact on MET. CD151 promoted proliferation, migration, and tube formation of HUVEC, accompanied by MET, FAK, and cdc42 activation. These effects were not seen using a HUVEC-CD151kd. In addition, CD151ko prevented high level expression of angiogenic molecules such as NO, CD106, and VEGF. The data suggest that CD151 supports angiogenesis possibly related to MET [155]**.**

4.4 In this study, the authors investigated whether CD151 promotes neovascularization and improves ventricular function after myocardial infarction in rats and the mechanisms involved. Rats were subjected to sham surgery or coronary artery ligation. The authors transfected rat myocardium with the human CD151 gene that was detected 4 weeks (wk) after coronary artery ligation. CD151-assisted PI3K/AKT and the GPCR eNOS activation but did not affect VEGF expression. The authors conclude that CD151 promotes neovascularization via PI3K/AKT without affecting ischemia-induced VEGF expression [158].

4.5 In this study the authors used HUVEC transfected with CD151 or CD151AAA to evaluate the prerequisite of CD151oe. HUVEC expressed CD151 and CD151AAA at a similar level. CD151oe promoted angiogenesis by ERK, FAK, and p130Cas activation depending on the association of CD151 with α3. None of these effects were seen with CD151-AAA transfected HUVEC. The authors conclude that proliferation, migration and tube formation essentially depend on the CD151-α3 complex that is crucial in FAK and p130Cas signaling. They suggested that disruption of the CD151-α3 complex may impair overshooting angiogenesis of metastasizing tumors [161].

4.6 The authors evaluated the impact of CD151-integrin complexes in lateral ischemic hindlimb using CD151 and CD151-ARSA mutant transfection that are both well expressed at the protein level. CD151, but not CD151-ARSA promoted angiogenesis and improved capillary density, aortic EC proliferation and migration. CD151, but not CD151-ARSA activated FAK, ERK, PI3K/AKT/eNOS, and Rac1/Cdc42 signaling. The authors suggest that a CD151-integrin complex is likely a prerequisite for CD151-induced angiogenesis and signaling pathway activation [159].

4.7 EWI-F is a glycosylated type 1 transmembrane immunoglobulin that interacts with CD151. The authors explored whether this interaction affects the impact to CD151 on angiogenesis. VEGF-induced capillary tube-like formation was inhibited by TNFα and was associated with a rise in CD9P-1 mRNA. Accordingly, CD9P-1kd inhibited VEGF-dependent *in vitro* angiogenesis. A truncated form of CD9P-1 (GS-168AT2) is not associated with CD151 and CD151 is not transported to the cell surface. GS-168AT2 inhibits EC migration, proliferation, and angiogenesis. The authors conclude that the GS-168AT2 potently inhibits angiogenesis by downregulation of CD151, which provided the first evidence for the central role of CD9P-1 in angiogenesis [163].

4.8 Angiogenesis also is impaired by mutating CD151. The C-terminal cytoplasmic domain of CD151 contains an YRSL. Mutation of this CD151 YRSL motif (CD151-ARSA) attenuates angiogenesis. The authors show that both CD151 and CD151-ARSA gene delivery were capable of increasing the expression of CD151 at the protein level in HUVEC without significant difference in CD151 protein expression. However, only CD151, but not CD151-ARSA seconded HUVEC cell proliferation, migration, and capillary network formation accompanied by PI3K/AKT and ERK signaling. Thus, the CD151 YRSL motif of CD151 is required for the attachment of signaling molecules that play key roles in CD151-induced angiogenesis [155].

4.9 One study was concerned about TEX promoted angiogenesis, measuring enzymatic activity of ectonucleotidases CD39/CD73 and adenosine content of TEX. TEX carry enzymatically active CD39/CD73 and adenosine. TEX fostered adenosine receptor 2b (A2BR)-mediated polarization of macrophages toward an M2-like phenotype and enhanced secretion of angiogenic factors. Growth of EC was stimulated directly by TEX and indirectly via macrophage-reprogramming dependent on A2BR signaling. *In vivo*, TEX stimulated the formation of defined vascular structures and macrophage infiltration. These effects were not observed in A2BRko rats. The authors conclude that **t**hey provided the first evidence for adenosine production via A2BR. They suggest A2BR antagonism as a potential strategy to block TEX-induced angiogenesis [174].

4.10 Crosstalk between the nervous and vascular systems is important during development and in response to injury. The authors evaluated the role of the laminin-like axonal guidance protein netrin-1 for its involvement in angiogenesis and vascular remodeling. Netrin-1 up-regulated CD151 and VEGF concomitant with the activation of FAK, src, and Paxillin that induced EC proliferation, migration, and tube formation. The authors interpret their findings that Netrin-1 promotes angiogenesis through CD151-dependent FAK/Src/Paxillin signaling suggesting the existence of a netrin-1/FAK/Src/CD151 complex [160].

4.11 The authors explored the impact of CD151 on angiogenesis in CD151ko mice. Consistent with an EC deficiency, isolated CD151-null mouse lung EC showed diminished support for B16F10 adhesion and transendothelial migration, diminished B16F10-induced permeability, and diminished B16F10 adhesion to the ECM. Impaired angiogenesis in CD151ko EC was accompanied by diminished Src and AKT activation that was not accompanied by reduced EC permeability. In summary, impaired angiogenesis in CD151ko mice may be a sequel of impaired interaction with the ECM accompanied by a defect in src and AKT activation [156].

4.12 To further assess the functional implications of CD151-integrin associations, the authors evaluated the effect of a CD151 mAB that blocked integrin binding on EC functions. Anti-CD151 inhibited EC migration and modulated angiogenesis but did not affect neutrophil transendothelial migration. CD151 promotes HUVEC proliferation, migration, and tube formation, accompanied by increased phosphorylation of AKT and eNOS, leading to NO upregulation. The CD151 mAB attenuating the effects suggested that CD151 activates the PI3K/AKT pathway, confirmed by inhibitors of PI3K and eNOS attenuating CD151-induced EC proliferation and cell migration. The authors conclude that activation of PI3K/AKT accounts for CD151-induced EC proliferation and migration [173].

4.13 The authors evaluated whether CD151 regulates angiogenesis in HUVEC via MET activation and which additional

angiogenic molecules might be involved. They found that proliferation, migration, and tube formation of HUVECs were promoted by CD151 with activation of MET, FAK and cdc42 and increased NO, VCAM-1 and VEGF levels. The data suggested that CD151 could promote angiogenesis possibly related to MET and associated protein signaling [136].

These findings convincingly demonstrate the involvement of CD151 in angiogenesis. Beside MET, signaling via associated integrins and CAMS is frequent. Other findings based on the association with EWI-F or those observed with CD151 mutants add to the spectrum of CD151-assisted angiogenesis. The data are summarized in **Table 4**.

Section 5. VEGFR2 and angiogenesis

Having noted impaired angiogenesis in Tspan8ko and CD151ko mice accompanied by a decrease in angiogenic receptors [30] we became particularly interested in publications evaluating the underlying mechanisms. In the following sections we focus on publications referring to the angiogenic receptors. An overview of VEGF-VEGFR relationships summarizes this for the VEGFR subtypes. In brief, VEGFA regulates angiogenesis and vascular permeability by activating VEGFR1 and VEGFR2. VEGFC/VEGFD mainly regulate lymphangiogenesis via VEGFR3. VEGFR are distantly related to the PDGFR family; however, they are unique with respect to their structure and signaling system. They strongly stimulate the PI3K/AKT pathway toward cell proliferation, VEGFR2 the major signal transducer for angiogenesis, preferentially utilizes the PLCγ/PKC/MAPK pathway [180]. Regulation of VEGFR2 represents an important mechanism for the control of angiogenesis. For a summary overview we recommend reference [181]. We here present only a small selection of those exploring the underlying mechanism.

In advance, we start with a comment on osteopontin (OPN), a glycoprotein binding integrins, being beside other functions, involved in angiogenesis. We became interested in OPN as it is downregulated in Tsp8ko and CD151ko mice [32]. We present three examples evaluating the underlying mechanism.

5.1 The authors used a hemisection spinal cord injury (SCI) to unravel the contribution of OPN. OPN in the spinal cord was significantly increased three weeks (wk) after SCI. Besides affecting motor functions and neuropathic pain, the authors noted pronounced angiogenesis accompanied by increased VEGF expression and AKT phosphorylation within the spinal cord, all of which were curbed by an OPNkd. The authors conclude that OPN contributes to angiogenesis through VEGF and AKT [182].

5.2 The authors treated endothelial progenitor cells (EPC) with CM of human glioma SHG44 overexpressing OPN. OPN secreted by glioma cells accelerated EPC angiogenesis *in vitro*, including proliferation, migration, and tube formation. OPN induced activation of AKT and eNOS and increased NO production without affecting VEGF, VEGFR1, or VEGFR2 expression. Moreover, anti-avβ3, a PI3-K inhibitor and an eNOS inhibitor suppressed the OPN-mediated increase in NO production and angiogenesis. The authors conclude that OPN directly stimulates angiogenesis via avβ3/PI3K/AKT/eNOS/NO signaling [183].

5.3 The authors demonstrate the contribution of OPN to angiogenesis in HUVEC. OPN enhanced the expression of VEGF through AKT and ERK. In turn, OPN-induced VEGF activates PI3K/AKT and ERK1/2 as a positive feedback signal. Blocking the feedback signal by anti-VEGF or PI3K inhibitor or ERK inhibitor reduced the OPN-mediated effects on HUVEC with anti-OPN being more effective. The data show that OPN enhances angiogenesis directly through PI3K/AKT and ERK with VEGF acting as a positive feedback signal [184].

5.4 It is known that VEGFR2 activity can be regulated by post-translational modifications such as ubiquitination and acetylation. Here the authors evaluated whether VEGFR2 can be regulated by SUMOylation. They report that EC-specific deletion of SENP1 reduces pathological angiogenesis and tissue repair during hindlimb ischemia, and VEGF-induced angiogenesis in the cornea, retina, and ear. SENP1-deficient EC showed increased SUMOylation of VEGFR2 and impaired VEGFR2 signaling. SUMOylation at lysine 1270 retains VEGFR2 in the Golgi and reduces its surface expression,

Table 4. CD151 in angiogenesis.

Target	Objective	Requirement / Signaling	Ref
CD151	α6β1	α6β1 promotes angiogenesis via Src-PI3K	[57]
CD151	MET	MET promotes angiogenesis via FAK, CDC42 accompanied by NO, VCAM-1 and VEGF upregulation	[155]
CD151	MET	NO↑, VCAM-1↑, VEGF↑, MET (FAK, CDC42) activation	[155]
CD151	CD151ko	Reduced EC permeability, ECM interaction, Src↓, AKT↓	[156]
CD151	Infarct	PI3K↑ AKT↑ eNOS↑	[158]
CD151	ARSA mutant	Angiogenesis↓↓↓, signaling PI3K↓, AKT↓ ERK↓	[159]
CD151	Netrin	Netrin1 upregulates CD151 and VEGF via FAK/Src/Paxillin	[160]
CD151	α3	CD151-α3: ERK, FAK, p130Cas	[161]
CD151	EWI-F	CD151↓ inhibits TNFα and VEGF signaling	[163]
CD151	Integrin	FAK, ERK, PI3K/AKT/eNOS, Rac1/Cdc42 signaling	[155]
CD151	tail mutation	Angiogenesis↓↓↓, signaling PI3K↓, AKT↓ ERK↓	[164]
CD151	Ischemia, CD151 inject.	CD151 promotes angiogenesis via PI3K/AKT↑ and eNOS↑	[173]
CD151	A2BRko	CD39/CD73 induces angiogenesis, abolished by A2BRko	[174]

attenuating VEGFR2-dependent signaling. The results show that VEGFR2 is regulated by deSUMOylation during pathological angiogenesis and propose SENP1 as a potential therapeutic target for overshooting angiogenesis as e.g. in diabetes [185].

5.5 VEGFR2 signaling in EC is essential for developmental and reparative angiogenesis. Reactive oxygen species and copper (Cu) are involved in these processes. However, their inter-relationship is poorly understood. The authors show that the high affinity copper uptake protein 1 (SLC31A1) functions as a redox sensor to promote angiogenesis. SLC31A1-depleted EC showed reduced VEGF-induced VEGFR2 signaling and angiogenic responses. Mechanistically, SLC31A1 is rapidly sulfenylated at Cys189 in the cytosolic C terminus after stimulation with VEGF, which induced SLC31A1-VEGFR2 disulfide bond formation and co-internalization into EE, driving sustained VEGFR2 signaling. *In vivo*, EC-specific SLC31A1ko mice or CRISPR-Cas9-generated redox-dead SLC31A1-knockin mutant mice had impaired developmental and reparative angiogenesis. Thus, SLC31A1 at Cys189 oxidation promotes VEGFR2 internalization and signaling to enhance angiogenesis. The study uncovers an important mechanism for sensing reactive oxygen species through SLC31A1 to drive neovascularization [186].

5.6 Recent studies highlighted the importance of ubiquitinases in angiogenesis. The authors utilized RNA sequencing data of a mouse retinal development model from the GEO database that identified significant upregulation of the potential proangiogenic deubiquitinases Ubiquitin carboxyl-terminal hydrolase (USP11). They demonstrated that USP11 expression correlates with key pro-angiogenic genes and is significantly upregulated at both mRNA and protein levels in VEGF-treated HUVEC. USP11ko markedly inhibited angiogenesis, whereas USP11oe promoted angiogenesis. Mechanistically, USP11 binds to peroxiredoxin 2 (PRDX2), facilitating the removal of its histone H3 at lysine 36 (K63)-linked polyubiquitination, which promotes its translocation into the nucleus. This facilitates the concurrent nuclear translocation of MYC, a PRDX2-interactor that subsequently enhances the transcription of VEGFR2 and activates VEGFR2 signaling. The authors conclude that USP11 promotes angiogenesis by upregulating VEGFR2 through the PRDX2/MYC pathway, indicating that USP11 could serve as a potential target for clinical interventions in angiogenesis-related diseases [187].

5.7 The authors evaluated the impact of tumor necrosis factor receptor superfamily member 9 (CD137) signaling that promotes angiogenesis via VEGFR2 and explored the underlying mechanism. CD137ko in apolipoprotein E $(ApoE)^{-/-}$ mice significantly decreased neovessel density in atherosclerotic plaques and EC proliferation, migration, and tube formation. Activation of CD137 signaling increases VEGFR2 transcription and translation. CD137 signaling activates VEGFR2 (Tyr1175) phosphorylation and the downstream AKT/eNOS pathway. Inhibition of VEGFR2 by siRNA or XL184 (cabozantinib) and inhibition of AKT or an eNOS inhibitor remarkably abolished proangiogenic effects of CD137 *in vitro* and *ex vivo*. CM from CD137-activated EC and VEGFA had similar effects on EC, where CD137 promotes VEGFA secretion, while blocking CD137 attenuates VEGFA secretion. In conclusion, activation of CD137 fortifies sprouting angiogenesis by increased VEGFA secretion and the VEGFR2/AKT/eNOS pathway [188].

5.8 This study builds on the one cited in [185]. The authors show that advanced glycation end product (AGE) increases nuclear factor erythroid 2-related factor 2 (Nrf2) translocation to the nucleus and promotes VEGFR2 expression. AGE also increases the expression of sentrin/SUMO-specific protease 6 (SENP6), which de-SUMOylated VEGFR2 indicating a reduction in VEGFR2 accumulation in the Golgi and increased VEGFR2 transport from the Golgi to the cell membrane via the coatomer protein complex subunit beta 2 (COPB2). The authors find SENP1 downregulation and VEGFR2 hyper-SUMOylated in overshooting angiogenesis. A non-SUMOylated form of VEGFR2 rescues angiogenic defects. They conclude that VEGFR2 SUMOylation is promoted by SENP1 and propose SENP1 as a potential therapeutic target for undue angiogenesis [189].

5.9 The authors describe a crosstalk of bone morphogenetic proteins (BMP) and VEGFR2 signaling. They demonstrate that BMP family members regulate VEGFR2 and Notch signaling, and act via Tafazzin (TAZ)-Hippo signaling, demonstrated in C57/Bl6 mice and in a porcine myocardial ischemia model. BMP 2/4/6 were identified as EC-specific targets of VEGF. BMP2 modulates VEGF-mediated EC sprouting via Delta like Canonical Notch Ligand 4 (DLL4). BMP6 modulates VEGF signaling by regulating VEGFR2 expression and acts via Hippo signaling effector TAZ in nude mice. BMP6 is the first member of BMP family found to directly regulate both Hippo signaling and angiogenesis. It may serve as a target in pro/anti-angiogenic therapies [190].

5.10 The authors evaluated VEGFR2 downstream signaling and whether the equilibrium between these pathways is modulated by coreceptors and distinct isoforms of VEGF in age-related macular degeneration. They investigated the influence of rab GTPase activating proteins (RabGAP) on VEGFR2 signaling, EC tube formation, and migration. They demonstrate that members of the TBC1 domain family member 10A (TBC1D10) have opposite effects. Whereas TBC1D10A promotes angiogenesis accompanied by increased ERK1/2 signaling, TBC1D10B lowered angiogenesis and ERK1/2 and p38 signaling. Though TBC1D10A were shown before to play a role in angiogenesis, the authors for the first time demonstrated colocalization with VEGFR2 and TBC1D10 as well as opposing activities of TBC1D10A and TBC1D10B [191].

5.11 Endocytosis plays a crucial role in receptor signaling. VEGFR2 and its ligand VEGFA are fundamental in neovascularization. However, the role of endocytosis in VEGFR2 signaling remained to be explored; only internalization via the clathrin-mediated pathway being well established. The authors show that this pathway is the predominant internalization route for VEGFR2 only in the absence of ligand. Instead, VEGFA induces a new internalization itinerary for VEGFR2, the pathway of macropinocytosis, which becomes the prevalent endocytic route for the receptor in the presence of ligand. Macropinocytic internalization of VEGFR2, which mechanistically is mediated through cdc42, takes place through macropinosomes generated at ruffling areas of the membrane, macropinocytosis playing a crucial role in VEGFA-induced signaling. The authors conclude that these findings expand our knowledge on the endocytic pathways of VEGFR2 and suggest that VEGFA-driven internalization of VEGFR2 through macropinocytosis is essential for angiogenesis [192].

5.12 Clinical and animal studies implicate erythropoietin (EPO) and EPO receptor (EPOR) signaling being involved in both physiological and pathological angiogenesis. In the eye, the authors hypothesized that EPOR signaling is important in pathological

angiogenesis, tested in a rat model of oxygen-induced retinopathy. EPOR expression and activation was increased and activated EPOR localized to retinal EC at postnatal day 18, when pathological angiogenesis occurred. In human retinal microvascular EC, EPOR was up-regulated and activated by VEGF. A VEGFkd reduced p-EPOR and p-VEGFR2. VEGFR2-activated EPOR caused an interaction between p-EPOR and p-VEGFR2. An EPORkd reduced VEGF-induced EC proliferation in association with reduced p-VEGFR2 and p-STAT3. A VEGFR2kd abolished VEGFA-induced EC proliferation and p-VEGFR2, EPOR, and STAT3. The authors summarize that VEGFA-induced p-VEGFR2 activates EPOR and causes an interaction between p-EPOR and p-VEGFR2 to enhance VEGFA-induced EC proliferation by exacerbating STAT3 activation [193].

5.13 Autophosphorylation of VEGFR2 tyrosine 1173 (Y1173) is considered a focal point for its angiogenic signaling. The authors evaluated signaling molecule recruitment that accounted for phosphorylation of Y1173. They demonstrate that c-src directly through its src homology 2 (sh2) domain and indirectly via c-Cbl binds to phospho-Y1057 of VEGFR2. Activation of c-src kinase by a positive feedback mechanism phosphorylates VEGFR2 at multi-docking site. c-src also catalyzes tyrosine phosphorylation of Ras GTPase-activating-like protein IQGAP1 (IQGAP1) and acts as an adaptor to bridge IQGAP1 to VEGFR2. IQGAP1 activates proto-oncogene B-Raf (b-Raf) and mediates EC proliferation. Silencing IQGAP1 and b-Raf revealed their activity being essential for VEGF-stimulated angiogenesis. The authors conclude that recruitment and activation of c-src is pivotal in angiogenic signaling of VEGFR2 by phosphorylation at Y1173 that facilitates association and activation of IQGAP1, critically required for EC proliferation [194].

5.14 TEX containing Tspan8 efficiently induces angiogenesis in tumors and tumor-free tissues. Little information exists on Exo-EC interactions and the proangiogenic role of exosomal tetraspanins, this study used a rat pancreatic adenocarcinoma (PDAC) to explore the effects of Tspan8-TEX on angiogenesis. Tspan8 contributed to a selective recruitment of proteins and mRNA into TEX, including CD106 and α4, which are implicated in TEX-EC binding and -internalization. TEX uptake induced VEGF-independent regulation of several angiogenesis-related genes, including von Willebrand factor (vWf), Tspan8, CXCL5, macrophage migration inhibitor factor (MIF), C-C chemokine receptor type (CCR)1, and, together with VEGF, VEGFR2. EC uptake of Tspan8-α4 complex-containing TEX was accompanied by enhanced EC proliferation, migration, sprouting, and maturation of EC progenitors. Taken together, TEX-initiated EC proliferation by upregulation of angiogenesis-related proteins may provide new options for therapeutic interference with overshooting angiogenesis [38].

We want to remember that with the abundance of publications on VEGFR2 signaling, we selected for a few articles, where either the VEGFR2 was inherently or experimentally altered or where angiogenesis was affected without knowing the involved molecules and signaling. It is notable that such a large array of proteins is involved in VEGFR2 activity. Data are summarized in **Table 5**.

Section 6. VEGFR3 and lymphangiogenesis

We recommend reference [195] for an overview on signaling via VEGFR3.

Our interest in lymphangiogenesis derived from the observation that a rat PaCa exclusively metastasizes via the lymphatic system [196]. Clinical studies in humans confirmed the involvement of VEGFR3 [197]. We will describe few of those examples elaborating pathways involving VEGF3 signaling.

6.1 One review article describes the main pathways associated with lymphangiogenesis highlighting the involved molecules. The pathway-related molecules of the VEGFC/D-VEGFR3/Neuropilin (NRP)2 axis involve furin-like enzyme, Contactin (CNTN)1, Prox1, LYVE-1, Podoplanin (PDPN), TF SOX18 (SOX18), SDF1 and CXCR4 that are direct constitutors as a portion of the VEGFC/D-VEGFR3/NRP2 axis, their biological activities relying on this ligand-receptor system. These axis-related signal molecules could gradually produce waterfall-like cascading effects, mediate differentiation and maturation of lymphatic (L)EC, remodel original and neonatal LEC, as well as ultimately promote tumor cell chemotaxis, migration, invasion and metastasis to lymphoid

Table 5. VEGFR2 signaling in angiogenesis.

Target	Effector	Activity	Ref
VEGFR2	Sumoylation	Promoted by SENP1, VEGFR2 retained in Golgi, angiogenesis↓	[185]
VEGFR2	SLC31A1	Cointernalization by disulfide bonds, VEGFR2 signaling↑, angiogenesis↑	[186]
VEGFR2	USP1oe	Binds PRDX2, removal histone H3-linked polyubiquitination, concurrent MYC nuclear translocation, VEGFR2↑ VEGFR2 signaling↑, angiogenesis↑	[187]
VEGFR2	CD137	VEGFR2 transcription, translation, activation↑ via AKT/eNOS, VEGFA↑ Angiogenesis↑	[188]
VEGFR2	SENP1ko	VEGFR2 SUMOylation↓ VEGFR2 signaling↑, angiogenesis↑	[189]
VEGF	BMP 2/4/6	Target: VEGF, BMP2 acts via DLL4. BMP6 regulates VEGFR2 via Hippo signaling TAZ, angiogenesis↑	[190]
VEGFR2	TBC1D10A	Act on RAB13, Erk1/2↑, VEGFR2 signaling↑ angiogenesis↑	
	TBC1D10B	ERK1/2↓, p38↓, VEGFR2↓, NRP1↓, angiogenesis↓	[191]
VEGFR2	Macropinocytosis	VEGFR2 internalization via macropinocytosis through Cdc42, angiogenesis↑	[192]
VEGFR2	EPOR	VEGFR2p induced EPORp and interaction via STAT3→pathol. angiogenesis↑	[193]
VEGFR2	c-SRC, IQGAP1	c-Src kinase via SH2 and c-Cbl promotes multiple VEGFR2p and catalyzes IQGAP1p bridging via b-Raf IQGAP1 to VEGFR-2p; angiogenesis↑	[194]
VEGFR2	Tsp8 TEX	Upregulation of vWf, Tsp8, CXCL5, MIF, CCR1, together with VEGF VEGFR2	[38]

tracts. The authors summarize the structure and function features of pathway-related molecules of VEGFC/D-VEGFR3/NRP2 axis, the expression changes of these molecules in different anatomic organs or histopathologic types or development stages of various tumors, the characteristics of transduction, implementation, integration of signal networks, the interactive effects on biological behaviors between tumor cells and LEC and their molecular mechanisms as well as the significance in tumor lymphangiogenesis and lymphatic metastasis [198].

6.2 Notoginsenoside R1 (R1) is a plant product that was frequently used as a therapeutic in China. In this study the authors investigated the therapeutic effect in a mouse model of tail acquired lymphedema and explored the underlying mechanisms. C57BL/6J mice and LEC specific VEGFR3ko transgenic mice underwent surgical induction of tail acquired lymphedema. R1 treatment mitigated lymphedema and enhanced VEGFC expression on LEC. The effects were abolished by a VEGFCkd and VEGFR3 inhibitors. A VEGFR3ko in LEC completely blocked R1 promoted lymphangiogenesis and lymphatic drainage. R1 activates the cAMP/PKA signaling pathway, leading to PKA and TF CREB phosphorylation. A PKA inhibitor and CREB siRNA inhibited R1-induced VEGFC expression. Additionally, R1 activated VEGFC promoter activity in a CREB-dependent manner. The authors conclude that R1 is the first reported small natural compound to promote VEGFC expression via cAMP/PKA/CREB signaling. The findings suggest R1 as a novel oral medication for patients with acquired lymphedema [199].

6.3 Lymphatic capillaries develop discontinuous cell-cell junctions that permit the absorption of large macromolecules, chylomicrons, and fluid from the interstitium. The authors asked whether and how VEGFR3 can alter lymphatic junctions. They used lymphatic-specific Flt4ko mice to investigate VEGFR3 signaling in lymphatic junctions. Loss of Flt4 prevents specialized button junction formation in multiple tissues and impairs interstitial absorption. A FLT4ko in human LEC resulted in impaired NOTCH1 expression and activation. Overexpression of the NOTCH1 intracellular domain in Flt4ko vessels rescued the formation of button junctions and absorption of interstitial molecules. The authors conclude that there is a requirement for VEGFR3 and NOTCH1 signaling in the development of button junctions in LEV that may hold clinical relevance to lymphatic diseases with impaired VEGFR3 signaling [200].

6.4 The authors show that chemokine signaling through CXCL12 and CXCR4 plays crucial roles in regulating LEC development. LEC-specific CXCRko mouse embryos and CXCL12 mutant embryos exhibit severe defects in lymphatic sprouting, migration and lymphatic valve formation. Deletion of CXCR4 or blockage of CXCL12 and CXCR4 activity results in reduced VEGFR3 levels on the LEC surface, which impairs VEGFC-mediated VEGFR3 signaling and downstream PI3K/AKT activities. The authors concluded that they uncovered a previously unknown mechanism by which CXCR4 modulates VEGFC, VEGFR3, and AKT signaling [201].

6.5 Ang and their Tie receptors regulate LEC development, and mutations of the Ang2 gene were recently found in human primary lymphedema. To unravel the mechanistic basis of Ang2 activity in lymphangiogenesis the authors used gene deletion, blocking mAB, transgene induction, and gene transfer to study how Ang2, Tie1, and Tie2 receptor regulate LEC. They discovered that VEGFC-induced Ang2 secretion from LEC is involved in PI3K/AKT activation. Neonatal deletion of genes encoding Tie receptors or Ang2 in LEC, or administration of an Ang2-blocking mAB decreased VEGFR3 presentation on LEC and inhibited lymphangiogenesis. A similar effect was observed in LEC upon deletion of the PI3K catalytic p110α subunit or with small-molecule inhibition of a constitutively active PI3K located downstream of Ang2. The authors conclude having uncovered an important crosstalk between the VEGFC and Ang signaling and suggest manipulation of lymphangiogenesis by targeting Ang2/Tie/PI3K signaling as new therapeutic avenues [202].

6.6 It is known that Prospero homeobox protein 1 (PROX1) is necessary for the development of LEC, lymphatic valves (LV) and lymphovenous valves (LVV). The authors previously noted a feedback loop between PROX1 and VEGFC signaling. PROX1 promotes the expression of VEGFR3 in LEC. In turn, VEGFC signaling maintains PROX1 expression in LEC. In the present study the authors evaluated the mechanism of the PROX1/VEGFC feedback and whether VEGFC signaling is necessary for LV and LVV development. They report that VEGFC signaling is necessary for valve morphogenesis and that the transcriptional co-activators YAP and TAZ are required to maintain PROX1 expression in LV and LVV in response to VEGFC signaling. Deletion of Yap and TAZ in the lymphatic vasculature of mouse embryos resulted in the degeneration of LVs or LVVs. The results identified VEGFC, YAP and TAZ as a crucial molecular pathway in valve development [203].

6.7 The authors explored how VEGFR3 signaling can be attenuated to prevent LEC overgrowth. They showed that ILK depletion in mouse embryos hyper-activates VEGFR3 signaling and leads to overgrowth of the jugular lymph sacs/primordial thoracic ducts, edema and embryonic lethality. LEC-specific deletion of ILK in adult mice initiates LEC expansion. An ILKko in human LEC triggers VEGFR3 tyrosine phosphorylation and proliferation. Furthermore, ILK impedes the interaction between VEGFR3 and β1, and EC-specific deletion of an β1 allele rescues the excessive LEC growth observed upon ILK depletion. Mechanical stimulation disrupts the assembly of ILK and β1, releasing the integrin to enable interaction with VEGFR3. Thus, ILK facilitates VEGFR3 signaling via controlling its interaction with β1 that ensures proper LEC development [204].

6.8 The authors report that an ischemia-inducible Gβγ signal regulator, activator of G-protein signaling 8 (AGS8) regulates VEGFR3 in human dermal (D)LEC. VEGFC stimulates DLEC proliferation and tube formation, inhibited by AGS8kd that affected VEGFC-mediated phosphorylation of VEGFR3 and downstream molecules ERK1/2 and AKT. Endocytosis inhibitors not rescuing the decrease of cell-surface VEGFR3, suggested that AGS8 regulates trafficking of VEGFR3 to the plasma membrane, where it forms a VEGFR3/AGS8/Gβγ complex. The authors conclude having unraveled a novel regulation of VEGFC-VEGFR3 by AGS8 [205].

6.9 The initial steps of lymphangiogenesis still being elusive, the authors found that the surface glycoprotein MUC18 (CD146) plays an important role. MUC18 acts as a receptor of VEGFC in regulating lymphangiogenesis, especially at the sprouting step by selective activation of downstream p38 mitogen-activated protein kinases (p38) and ERK upon VEGFC stimulation. The authors uncovered a critical role of MUC18 in initiating VEGFC signaling in lymphangiogenesis [206].

Even taking into account the abundance on information about signaling via VEGFC/D and VEGFR3 (reviewed: [195,198]), we felt it important to cite at least a few articles that report on treatment options in overshooting lymphangiogenesis taking into account that lymphedema is a major problem. The good news is that there is convincing medication to interfere with lymphedema.

Finally, as EphA4 decreased in Tspan8ko and CD151ko, we wanted briefly commending on the engagement of Ephrins in angiogenesis. We recommend a review on EphR in angiogenesis [207]. We also want to stress that Ephrins are a hub for signaling and endocytosis [208].

We present some examples.

6.10 The authors show that ephrinB2, a transmembrane ligand for EphA4E, promotes sprouting and motility in the angiogenic endothelium. The authors could link this pro-angiogenic function to a crucial role of ephrin-B2 in VEGFR3 signaling. In the absence of ephrinB2, the internalization of VEGFR3 is defective, which compromises downstream signal transduction by rac1, AKT, and Erk1/2. The authors conclude that full VEGFR3 signaling is coupled with receptor internalization, where EphrinB2 acts as a key regulator controlling both angiogenesis and lymphangiogenesis [209].

6.11 Leptomeningeal anastomoses or pial collateral vessels play a critical role in cerebral blood flow (CBF) restoration following ischemic stroke. The magnitude of this adaptive response is postulated to be controlled by the endothelium. The authors used endothelial genetic deletion of EphA4fl/fl/Tie2-Cre and EphA4fl/fl/VeCadherin-CreERT2 mice. They observed EphA4 as a major suppressor of pial collateral remodeling that is limited by the crosstalk between EphA4-Tie2 signaling in VEC mediated through p-AKT regulation. Peptide inhibition of EphA4 resulted in acceleration of the pial arteriogenic response. The authors demonstrate that EphA4 is a negative regulator of Tie2 and conclude that targeting of EphA4 and/or Tie2 represents an attractive new strategy for functional recovery following ischemic stroke [210].

6.12 The authors were interested whether EpHA4 has an impact on adhesion that they elaborated by binding of monocytes to EC using besides others atomic-force microscopy based single-cell force spectroscopy. They observed a proadhesive effect of ephrinB2 being independent of active transcription and mediated via rho signaling with subsequent modulation of the actin cytoskeleton. EphrinB2 was induced by TNFα treatment and silencing of ephrinB2 lowered TNFα mediated monocyte adhesion to EC. The authors stress their findings pointing to a crucial role of ephrinB2 induced EphA4 forward signaling in the context of cell adhesion to EC, the transcription-independent effect being mediated by rho signaling induced actin-filament polymerization [211].

6.13 Neurobiological consequences of traumatic brain injury (TBI) result from a complex interplay of secondary injury responses and sequela that mediates chronic disability, where EC are important regulators of the cerebrovascular response to TBI. The authors demonstrate that EC-specific EphA4 deletion in ko mice promotes blood-brain barrier (BBB) integrity and tissue protection. Sequencing of EphA4ko EC showed increased differential gene expression of BBB-related junctional and actin cytoskeleton regulator A-kinase anchor protein 12 (Akap12), whose presence at Tie2 clustering domains is enhanced in ko microvessels. Transcript and protein analysis of cortical EC suggest that EphA4 limits the expression of claudin (Cldn)5, AKT, and Akap12 and fortifies Ang2. Blocking Tie2 reversed Akap12 expression. Direct stimulation of Tie2 phenocopied the neuroprotection. Finally, the authors noted a rise in soluble Ang2 in the sera of individuals with acute TBI, highlighting its promising role as a vascular biomarker for early detection of BBB disruption. Thus, EphA4 strengthens TBI microvascular dysfunction through negative regulation of Tie2/Akap12 [212].

Taken together, even with these few examples it becomes obvious that EpHA4 acts via its hub activity and by its many ligands also is involved in lymphangiogenesis.

Data are summarized in **Table 6**.

Table 6. VEGFR3 and lymphangiogenesis.

Target	Effector	Activity	Ref
VEGFC	Notoginsenoside	Activates cAMP/PKAp and CREBp, CREBp promotes VEGFC transcription	[199]
VEGFR3	NOTCH1	Lymphatic junctions require FLT4 and NOTCH1	[200]
VEGFR3	CXCL12/CXCR4	Deletion/blockade of CXCL12/CXCR4→VEGFR3↓ (PI3K/AKT↓)	[201]
VEGFC	Ang2, Tie2	VEGFC→Ang2↑ (AKT/PI3K) →VEGFR3↑, Ang2/Tie blocking: VEGF/VEGFR3↓	[201]
VEGFC	PROX1	PROX1/VEGFC feedback loop. VEGFC via YAP and TAZ maintain PROX1	[203]
VEGFR3	ILKko/kd	ILKko lethal, ILKkd VEGFR3p↑↑↑; ILK controls VEGFR3p interaction with β1	[204]
VEGFR3	AGS8	AGS8→VEGFR3↑ ERK1/2↑, AKT↑, regulates VEGFR3 traffic to the membrane, where it forms a complex with AGS8 and Gβγ	[205]
VEGFC	MUC18	MUC18 (VEGFC receptor) upon VEGFC stimulation regulates lymphatic sprouting via p38 and ERK	[206]
VEGFR	Ephrin-B2	Required for VEGFR3 internalization via Rac1, AKT and Erk1/2, (lymph) angiogenesis↓	[209]
LEC	EphA4	EphA4 is a negative regulator of Tie2 through p-AKT. Targeting EphA4/Tie is suggested to improve lymphangiogenesis/functional recovery after stroke	[210]
Monocytes	EphA4/Ephrin B2	A proadhesive effect of ephrinB2 is mediated via Rho promoting actin cytoskeleton modulation by TNFα	[211]
Cortical EC	EphA4	EphA4 limits Cldn5, Akt, Akap12 A expression, promotes Ang2; EphA4 mediated TBI dysfunction via Tie2/Akap12	[212]

Last, not least, we should at least mention that many of the cited studies elaborated the impact on angiogenesis using sExo or TEX. For an overview we recommend reviews cited in [213–215], additional examples are cited in [215–219]. The reference [162] and references cited in [123–128,150,174] used TEX. We used sExo/TEX in the studies cited in [30–32,38,79,155,175] and provide an overview on TEX in [220].

Section 7. Therapeutic interference via Tsp8 and CD151

In advance we have to admit that we only cite selected examples that approved their results *in vitro* or *in vivo*. We will start with the impact of mAB, recommending ref [176,221–223] for an overview.

Therapeutic interference with mAB

7.1 The authors describe that inhibition of HEp3 (hepatoma line) metastasis by a CD151 mAB could not be attributed to any effect of the mAB on tumor cell growth *in vitro* or *in vivo*. Instead, the mAB inhibits metastatic foci formation [224].

7.2 The authors describe that a CD151 mAb prevents tumor cell dissemination by inhibiting intravasation without affecting primary tumor growth, proliferation, and extravasation [225].

7.3 CD151 mAB F(ab')2 fragments cause homotypic adhesion of HEL and K562 dependent on cytoskeleton integrity. The adhesion was not blocked by β1 or β3 mAB, Instead, CD151 acts as a component of integrin signaling [226].

7.4 Several CD151 mAB display anti-metastatic activity in vivo that was essentially attributed to cell motility inhibition. The authors generated new CD151 mAB that directly inhibit tumor growth in xenograft cancer models and metastasis in orthotopic cancer models [227].

7.5 The authors generated a CD151 mAB that prevents the association with α6β1. It detects CD151 in the membrane and cytoplasm of hepatocellular carcinoma (HCC) cells, as well as in liver cells. *In vitro* assays demonstrated that the mAB inhibits neoangiogenesis and HCC mobility and invasiveness. In vivo the mAb inhibited HCC metastasis [228].

7.6 The authors evaluated the impact of a Tspan8 mAB on SW480 (CRC) *in vitro* and *in vivo* (s.c.). By early treatment, the Tsp8 mAB inhibited growth of Tspan8 expressing tumors, not linked to toxicity or to angiogenic properties. *In vivo*, it inhibited cell proliferation. The finding differs from those described using the same mAB *in vitro* suggesting that *in vivo* mAB binding modifies the response to signals issued from the tumor microenvironment (TME). Given the restricted expression of Tspan8, these preliminary results demand further investigations [229].

7.7 GEM and a Tspan8 mAB retard A81.8 (PaCa) tumor growth and prevent metastasis, with the Tspan8 mAB being more effective than GEM. Tspan8 mAB and GEM act via different pathways. GEM preferentially suppressed myeloid-derived suppressor cells (MDSC), only Tspan8 strongly inhibited VEGFR3 expression in nude mice [230].

7.8 A Rat PaCa expressing Tspan8 induces lethal disseminated intravascular coagulation, suggesting Tspan8 engagement in angiogenesis. Without affecting tumor growth, angiogenesis was completely inhibited by the Tspan8 mAB, irrespective of the tumor expressing Tspan8. This is the first report that Tspan8 induces systemic angiogenesis, the mAB with high selectivity affecting sprouting EC. Similar effect were observed by an α3 mAB [175].

7.9 The authors validated Tspan8 as a potential therapeutic target in metastatic (m)CRC. They identified the LEL as a key domain for regulating mCRC invasion. They developed a novel anti- Tspan8-LEL human antibody that specifically recognizes amino acids (AA) 140–205 of Tspan8-LEL. The antibody specifically reduced invasion of mCRC cell lines, which show high Tspan8 expression. The data uncovered that Tspan8-LEL plays an important role in mCRC invasion and suggest evaluating the efficacy to the antibody *in vivo* [231].

7.10 Tspan8 being overexpressed in CRC with a poor prognosis, the authors used a radiolabeled Tspan8 mAB for screening of potential metastases. The antibody very selectively recognized Tspan8 overexpression that correlated with poor prognosis. They repeated the experiment applying radiolabeled Tspan8 mAB in nude mice bearing CRC xenografts with high Tspan8 expression. The antibody was localized with high specificity to the tumor tissue and was well retained. The authors noted a significant inhibition of tumor growth in mice treated with the radiolabel led mAB and suggest its use in patients with aggressive Tspan8-positive CRC [232].

7.11 In this study the authors found that the Tspan8-LEL is a key regulator of ovarian cancer (OC) invasion. The Tspan8-blocking mAB promotes internalization and concomitant downregulation of surface Tspan8. Monotherapy with a Tspan8 mAB shows that mAB-based modulation of Tspan8-LEL significantly reduces the incidence of OC metastasis without severe toxicity *in vivo* [233].

Other studies approached the impact of CD151 via its associated miR-214-3p.

7.12 This study probed the effect of targeted regulation of CD151 by miR-214-3p delivered by BM stem cell (BMSC) sExo in Alzheimer's disease (AD). AD rats had prolonged escape latency, weakened learning and cognitive ability, increased neuronal apoptosis in the hippocampal CA3 region, and aggravated oxidative stress. After MSC-sExo injection, these changes were partially rescued. Thus, miR-214-3p targeted CD151 improved AD by enhancing antioxidant capacity [234].

7.13 The authors wanted to elaborate the molecular mechanisms that accounted for heart failure (HF). They found increased miR-124 cardiac expression in patients and mice with HF and identified CD151 as a direct target of miR-124. miR-124 over-expression aggravated Ang II infusion-induced cardiac dysfunction and abnormal cardiac angiogenesis in mice. *In vitro*, transfection of miR-124 mimics reinforced apoptosis and reduced viability, migration, tube formation, and NO in EC. The authors suggest that miR-124 is an important negative regulator of cardiac angiogenesis and cardiac function by suppressing CD151. They recommend modulation of miR-124 as a new strategy for HF therapy [177].

7.14 Cyclo L-Leucyl-L-Prolyl peptide (CLP) is a marine natural metabolite. The current study aimed to determine the effect of CLP on migration and growth of BC. CLP reduced the viability of BC cells but not breast healthy epithelium. CLP inhibited proliferation and migration, induced DNA strand breaks and cell death. *In silico* docking showed preferential interaction with CD151 and significantly reduced membrane-bound CD151. CLP reduced the interaction of CD151 with EGFR. The authors conclude that CLP

Table 7. Therapeutic interference via Tsp8 and CD151.

Inhibitor	Question	Target	Mechanism/Impact	Ref
CD151 mAB	Metastasis	Hep3	Impaired migration	[224]
CD151 mAB	Dissemination	Hep3	Impaired intravasation	[225]
CD151 mAB (F(ab')2	Cytoskeleton	HEL/K562	Blocked integrin signaling	[226]
CD151 mAB (new)	Metastasis		Direct inhibition *in vitro* and *in vivo*	[227]
CD151 mAB not binding α6β1	Metastasis	HCC	Inhibit growth (38%), metast (83%)	[228]
Tsp8 mAB	*In vivo* growth	Tsp8+CRC	Crosstalk with TMA (70% inhib.)	[229]
Tsp8 mAB versus GEM	Mode of action	A818/nude	Tsp8: VEGFR3↓, GEM: MDSC ↓	[230]
Tsp8 mAB	c-coagp	EC	Tsp8-induced EC sprouting↓	[175]
Tsp8-AA140-205 mAB	CRC invasion	HCT116	Metastasis inhib, mechanism?	[231]
Tsp8 mAB, radiolabeled	Radiotherapy	HT29 /nude	High efficacy of radiolabeled Tsp8	[232]
Tsp8 mAB	Metastasis	OV cancer	Metastasis↓ by Tsp8 internalization	[233]
miR-214-3p /CD151	AD treatment	Exo in AD	Targeting CD151 improves AD by enhancing antioxidant capacity	[234]
miR-214-3p /CD151	Heart failure	Heart tissue	Restored CD151 improves cardiac function by NO↑, eNOS↑	[177]
CLP	CLP activity	BC	CLP affects growth via EGFR and CD151 signaling	[177]
SMCC – Tsp8	Reprogramming	BC	Tsp8↓↓, tumorigenicity↓, drug sensitivity↑ due to Tsp8 stemness	[235]
Drugs targeting CD151	PCL, FU	BC	Bind CD151-LEL, reduce viability, induce G1 arrest and apoptosis	[236]
Cotreatment irradiation / Tau	RT resistance	BC	Cotreatment migration↓, apoptosis↑, caspase 3↑, well suited for RT BC	[237]
lncRNA PVT1/miR-152	miR-152/CD151	GC	PVT1 inhibits miR-152, CD151↑, correlates with cancer progression	[241]
lncRNA SOX21-AS1	Link to Tsp8	LC	SOX21-AS1 interacts with GATA6 binds Tsp8 promoterà Tsp8↑ (oncogenic activity)	[242]
GPCR CD97 (high expr.)	Lymphatic metast.	GC, ifp	Accumulation in dr. LN promoting lymphatic metastasis	[245]
CAR-T (CD318, Tsp8)	Therapeut. impact	PaCa	*In vivo* cure: CD318 CAR-T after 19d, Tsp8 CAR T after 27d	[250]

suppresses growth and migration by attenuating cell cycle of BC via EGFR and CD151 signaling. They suggest that CLP may provide a new approach in the treatment of BC [177].

7.15 Tumor cells can reduce malignancy and even restore the function of benign cells through reprogramming. In this study, BC rapidly were reprogrammed by a novel small-molecule compound cocktail (SMCC) consisting of DAPT, dexamethasone (DEX), SB431542, ascorbic acid, and forskolin. The cocktail significantly reduced proliferation, metastasis, tumorigenicity, and malignancy, while increasing drug sensitivity *in vitro* and *in vivo*. They found that the SMCC strongly reduced the expression of Tspan8 in BC cells. They conclude that the stemness of Tspan8 obviously plays a key role in SMCC induced reprogramming [235].

Therapeutic interference with drugs and/or combined therapy

7.16 The authors evaluated the efficacy of targeting drugs to CD151. Molecular docking study identified pyrocatechol (PCL) and 5-fluorouracil (FU) suitable for binding to CD151-LEL. These CD151 targeting drugs significantly reduced BC viability, induced G1 cell cycle arrest and apoptosis, but had no impact on normal healthy breast epithelial cells. The authors suggests particularly PCL as a potential therapeutic drug in BC [236].

7.17 Clinical trials demonstrated the potential of sensitizing radiation therapy (RT)-resistant BC through the combination of chemotherapy and RT. This study explored the potential of CD151 as a therapy response marker in the co-treatment strategy involving ionizing radiation (IR) and the antiviral drug 2-Thio-6-azauridine (TAU) for sensitizing RT-resistant BC. The results showed reduced cell migration, pronounced apoptosis and increased caspase 3 activity and significantly reduced CD151. The findings suggest the potential of combination treatment with IR and TAU as a promising strategy to overcome RT resistance in BC, where CD151 emerges as a valuable therapy response marker [237].

Therapeutic interference via lncRNA

lncRNA are longer than 200 nucleotides and have no coding potential. They can act as a scaffold by interacting with RNA binding factors; can recruit chromatin-modifying enzymes resulting in repression of certain genes; contribute to the spatial organization of the genome; guide TF to specific genomic loci to regulate gene expression, function as decoy by preventing interaction of proteins with their ligands, attack mRNA stability, affect signaling pathways, act as sponge for miRNA. Finally, it should be mentioned that disease-associated expression changes suggested a potential role for lncRNAs in driving cancer. However, more recent studies suggest that this link does not essentially hold true. It could be verified by lncRNA-ko that in many instances remains to be done. We recommend some reviews on the classification and functions of lncRNA [238–240].

We will present two examples that describe the linkage to CD151 and Tspan8.

7.18 The lncRNA PVT1 is up-regulated and miR-152 is decreased in GC. PVT1 has three binding sequences for miR-152

and inhibits the expression of miR-152 that is a target of CD151. PVT1 was positively associated with CD151 and FGF2 expression in GC tissues. Thus, PVT1 acts as a "sponge" to inhibit miR-152 in GC and is a promising target to improve GC diagnosis and therapy [241].

7.19 The authors describe that in LC lncRNA SOX21-AS1 interacts with GATA-binding factor 2/6 (GATA6), which binds to the Tspan8 promoter and promotes Tspan8 expression, which enhances colony formation, proliferation and invasion *in vitro* and growth and invasion of xenograft tumors in nude mice. Silencing of SOX21-AS1 or inhibiting its binding to GATA6 downregulates Tspan8 and thereby exerts an anti-oncogenic effects in LC. The authors conclude that SOX21-AS1 via GATA6 promotes Tspan8 transcription and that high Tspan8 expression obviously exerts oncogenic activity [242].

CXCR4 as therapeutic, CXCR4 being the most common target in cancer therapy, reviewed in [243,244].

7.20 The GPCR CD97 is involved in transmembrane signaling, its expression being highly increased in a GC cell line metastasizing via the lymphatic system. Exo enhanced cell proliferation and invasion. Intrafootpad (ifp) injections of TEX derived from the metastatic line strongly promoted tumor cell accumulation of the metastasizing and the non-metastasizing line in the draining LN. CD55, CD44v6, α5β1, CD31, EpCAM and CD151 expression was increased. Taken together, CD97, aided by TEX, promotes GC lymphatic metastasis and plays a pivotal role in premetastatic niche formation [245].

CAR-T based therapy

CAR-T cell is considered the most promising approach in cancer therapy [246–249].

We selected one outstanding article concerned about immunotherapy in PaCa.

7.21 A major roadblock prohibiting effective immunotherapy of PaCa relies on the missing information on suitable targets. The authors identified cutaneous lymphocyte antigen (CLA), CD66c, CD138 (syndecan 1), a heparin sulfate glycoprotein, and Tspan8 as possible target for CAR-T therapy. They designed a CAR library with scFv orientation, CD3ζ, the 4-1BB stimulatory domain and the CD8a transmembrane domain. CAR-T activity was tested *in vitro* with respect to killing. Promising candidates were tested in NOD scid gamma (NSG) mice with a subcutaneous human PaCa graft. When the tumor reached 25 mm^2 mice received $5x10^6$ control or CAR T cells. All tumors grew until 9d post T cell injection. All mice treated with control T cells died latest 13d after injection. All CD318-CAR-T receiving mice were tumor free by 19d after injection and Tspan8-CAR-T receiving mice by 27d after injection. Thus, CD138- and Tspan8-specific CAR T cells are very promising target candidates for PaCa therapy in humans [250].

Taken together, most therapeutic approaches achieved an impact on retarding tumor growth or undue angiogenesis that was frequently improved by concomitant application of chemotherapy or irradiation. Only the CAR-T based therapy was curative in 100% of mice, indirectly proving the oncogenic features of Tspan8. We may add that it was the most work-intensive approach. In view of the overwhelming therapeutic effect, clinicians aiming for cancer therapy should search for collaborators to take over the intense work for finding the most suitable CAR-T. Patients worldwide will be most graceful.

Conclusion and Outlook

This article aimed to support our previously published data on the impact of a Tspan8ko, a CD151ko and a dbko on tumor growth and progression (section 2), hematopoiesis (section 3), angiogenesis (sections 4-6) and therapy (section 7) by a search for publications that evaluated the underlying mechanisms as our publications remained at the descriptive level.

Section 1: Tspan8 and CD151 in sExo/TEX biogenesis and delivery includes (**Table 1)**.

Section 2a: Tspan8 and CD151 in tumor induction/growth [50–53].

Section 2b: Tspan8 and CD151 in adhesion and migration [58–60,63,64,81–83,60]

Section 2c: Tspan8 and CD151 in tumor progression [94–115]

Section 2d: Tspan8 and CD151 and proteases [81–83,97,116–120]

The criss-cross evaluation of wt, Tspan8ko, CD151ko and dbko tumor and host cells provided evidence for an impact on tumor cell dissemination. Searching for mechanisms whereby Tspan8 or CD151 uncovered an impact on tumor induction and growth being due to defects in cooperation with adhesion molecules, proteases, RTK and GPCR. Metastasis-promoting Tspan8 and CD151 activities proceed at multiple levels, where Tspan8 and CD151 cooperate with different partners in distinct cell populations frequently involving associated adhesion molecules and proteases. Tspan8 and CD151 being unexpectedly upregulated in sExo and TEX [58–60,63,64,81–83,60,94–120], explains their strong impact on tumor growth and metastasis. We want to mention that section 2b and section 2c cannot be strictly separated. We followed the separation according to the authors' point of view. Data are summarized in **Tables 2a–2d**.

Section 3: Tspan8 and CD151 TEX in the crosstalk with HSC.

TEX-promoted deviation of early hematopoietic progenitor maturation rests, at least in part, on Tspan8 or CD151 associations with RTK [131–141] and GPCR [143–146]. Furthermore, wt TEX facilitate tumor cell recruitment and settlement in the BM by associated adhesion molecules and chemokine receptors [142–145]. Data are summarized in **Table 3**.

Sections 4: Tspan8 and CD151 in the crosstalk with EC.

Angiogenesis is affected in CD151ko and more strongly in Tspan8ko EC, Tspan8ko also affects lymphangiogenesis. We commemorate some examples on the impact of CD151. Defects in ko EC are corrected by sExo and repair is boosted by TEX. CD151 strengthens MET or MET and PI3K activation, blocking CD151 constrains MET, FAK, cdc42 activation. Dependent on the type of CD151 mutation angiogenesis is completely blocked or scr/AKT activation is impaired. Prohibiting the CD151-Netrin association curbs FAK/Src activation; interfering with the CD151-α3 association prohibits FAK/p130cas activation [57,136,155,156,158–161,163,167,173,174]. Data are summarized in **Table 4**.

Section 5: OPN [182–184] and VEGFR2 [38,185–195] in angiogenesis. Data are summarized in **Table 5**.

Section 6: VEGFR3 [198–206] and CXCR [209–212] in lymphangiogenesis. Data are summarized in **Table 6**.

Section 7: Therapeutic interference via Tsp8 and CD151

The section on therapeutic interference presents very few examples for mAB [224–233], miR [177,234], drugs [177,235,236], irradiation [237], lncRNA [241,242], CXCR4 [245] and CAR-T [250] based therapy. We need to stress that the efficacy of CAR-T with 100% cure is overwhelming.

Taken together, though much progress has been achieved, sometimes there is a certain bias due to the angle of view. It may be recommendable to check databases in advance not to oversee the range of parameters that should be taken into account. A very personal comment, as far as Tspan8 is expressed, take it as target as expression on non-transformed cell is very rare (except EC) that may help avoiding side effects. In addition, it functions as an oncogene and, in line with this, is expressed in CSC.

In view of the extraordinary role of Tspan8 in health and disease, we have added **Table 8** summarizing the Tspan8 engagement in tumor induction and metastasis, including the impact on CSC, its engagement in benign diseases and in development (**Table 8**).

Finally, one has to mention that much progress has been achieved by new technologies in any field of medicine and biological sciences that improved life expectancy and life quality. Medicals and scientists should fight worldwide for the freedom of science to develop new techniques, which always include the risk of failure, and to incorporate new techniques into their work.

Table 8. Tspan 8 activities.

Activity	Target	Pathway	Ref
Cancer			
CSC induction (PaCa)	NCSC	Tsp8-TEX activate SHH signaling→ conversion of NCSC into CSC	[251]
CSC induction (CRC)	NCSC	Stemness associated transcriptomic program: Tsp8↑ in metastasizing CRC	[252]
Tspan8 oncogene (multiple Ca)	STAT3	Tsp8 interacts with STAT3->chromatin occupany↑-transcription of cancer promoting genes↑↑, mAB blocking Tsp8 internalization prevents cancer gene transcription	[253]
Tsp8 oncogene (PaCa)	SOX9	SOX9 transcription regulator of Tsp8, EGF/SOX9/Tsp8 signaling Tsp8↑	
Tsp8-melanoma	Tsp8 transcription	Tsp8-β-catenin positive nuclear feedback loop→Tsp8↑↑, →Tsp8 oncogene -->melanoma invasion↑↑	[64]
Tsp8-melanoma	Tsp8 transcription	Tsp8-β-catenin positive nuclear feedback loop→Tsp8↑↑→ melanoma metastasis↑	[263]
Tsp8 melanoma	LCMR1	Tspan8 target of LCMR1, activation of RAF/MEK/ERK signaling→metastasis↑↑	[255]
Tsp8-TEX	Liver, lung, spleen	Tsp8-TEX ready uptake *in vivo* in liver, lung, spleen→forces motilitytumor spread↑	[256]
Tsp8-HCC	ADAM12m	Tsp8→ADAM12m↑→ HCC progression and metastasis↑	[257]
Tsp8-OC	Tsp8-LEL	mAB blocking Tsp8-LEL→Tsp8 internalization, surface expression↓ metastasis↓	[95]
Tsp8-glioma	Tsp8 high	Tsp8/FAK complex→proliferation↑, migration↑, drug-resistance↑	[258]
Tsp8-glioma	Tsp8/α3/rictor	Tsp8/α3/rictor complex, rictor being a key component mTORC2; Tsp8/rictor→ mTORC2 assembly, AKT/PKCα↑ kd of anyone of the 3 proteins→ migration↓	[259]
MUC1-GC	Tsp8	MUC1kd→Tsp8↓→proliferation↓, apoptosis↓, aggregation↓	[260]
Tsp8oe-GC	EGF	Tsp8oeEGF signaling↑→proliferation↑, invasion↑	[261]
Tsp8-GC	GC/non-transform.	GC sequencing: Tsp8↑, invasion↑, angiogenesis↑, prognostic classifier	[262]
Tsp8-GC	Gene expr survey.	SSH of GC: Tsp8↑↑	[266]
Tsp8-PrCa	AR	Tsp8 mutual deubiquitination AR→complex stability↑, nuclear Tsp8 corecruited AR promoter, required for expression→AR drive PrCa progression	[267]
Tsp8 in benign disease			
Multiple diseases	Tsp8	CNV in Tsp8 exon 11: disease-associated	[280–282]
Diabetes	Tsp8	Tsp8 type 2 diabetes associated	[283]
Glomerulonephritis	Tsp8	Tsp8 glomerulonephritis associated	[272]
Insulinoma	Tsp8	Tsp8 upregulated	[274]
Distorted coagulation	Tsp8/ADAMTS13	Tsp8 activates ADAMTS13, required for vWF activity	[284]
Impact on development			
Long lived quiescent MaSC	Foxp1/Tsp8	FoxP1ko: quiescent Tsp8hi MaSC↑→no exit from dormany to differentiation	[275]

Deeply quiescent MaSC	LGR5+Tsp8hi	postnatally quiescent, LGR5+Tsp8hi poised for activation in adults by ovarian hormones	[279]
Activation of MaSC	Foxp1/Tsp8	Foxp1 represses Tsp8 in basal cells, Tsp8 required for MaSC differentiation during development	[278]
Ovarian development	FOXL2/Tsp8	FOXL2 regulates Tsp8, FOXL2 facilitates exit of differentiating cells (coelomic epithelium), drives them towards a transitional identity, progressing into early supporting gonadal-like cells.	[277]
Spermatogonial SC	Tsp8hi vs.Tsp8lo	Tsp8hi enriched in SSC, correlates with hypomethylated domains in the promoter	[276]

Authors' Contributions

MZ wrote the draft. KZ and ZW read, corrected and approved the final version.

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