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Review

The role of yes activated protein (YAP) in melanoma metastasis

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SUMMARY

Hippo was first identified in a genetic screen as a protein that suppressed proliferation and cell growth. Subsequently, it was shown that hippo acted in a so-called canonical cascade to suppress Yorkie, the Drosophila equivalent of Yes-activated protein (YAP), a mechanosensitive transcriptional cofactor that enhances the activity of the TEAD family of transcription factors. YAP promotes fibrosis, activation of cancer-associated fibroblasts, angiogenesis and cancer cell invasion. YAP activates the expression of the matricellular proteins CCN1 (cyr61) and CCN2 (ctgf), themselves mediators of fibrogenesis and oncogenesis, and coordination of matrix deposition and angiogenesis. This review discusses how therapeutically targeting YAP through YAP inhibitors verteporfin and celastrol and its downstream mediators CCN1 and CCN2 might be useful in treating melanoma.

INTRODUCTION

The protein hippo was initially identified in *Drosophila melanogaster* using genetic screens that selected for mutations causing a cancer-like eye phenotype that was characterized by increased cell number and size, but decreased apoptosis (i.e., a hippopotamus-like phenotype.[1,2] Subsequent analyses identified a so-called hippo kinase cassette, consisting of four proteins: Hippo (Hpo), Salvador (Sav), Warts (Wts), and Mob-*as*-tumor-suppressor (Mats).[3] Hpo and Wts are serine/threonine kinases that are activated by phosphorylation, and act sequentially within the pathway. Biochemical studies of the Drosophila, and the related mammalian proteins, concluded that Hpo activation involves its autophosphorylation and the subsequent downstream phosphorylation of Sav, Wts, and Mats.[2,4–8] Sav acts as a scaffold to coordinate binding of Hpo and Wts into a protein complex.[2] Mats is an essential co-factor for Wts.[7] Wts activity is also regulated by Hpo-dependent phosphorylation, and its autophosphorylation.[6] Although multiple substrates for Hpo and Wts kinases have been identified, the pertinent target of Hpo, in the canonical hippo pathway, is Wts, and the pertinent downstream effector of Wts is the transcriptional co-activator Yorkie (Yki).[8] *yki* encodes the *Drosophila* ortholog of the mammalian protein yes-associated protein (YAP) (Figure 1).[9,10] Yki is required for normal tissue growth and is phosphorylated and inactivated by Wts. Human homologs of *wts* (i.e., *lats*), *hpo* (*mst2*), *mats* (*mats1*) and *yki* (*yap*) exist, and overexpression of these rescue the respective *Drosophila* mutants.[2,4–8] That is, the components of the hippo cascade are evolutionarily conserved.

Large tumor suppressor (Lats) is the mammalian equivalent of wts; Lats1/2 kinase mediated phosphorylation of YAP at ser127 promotes the binding of 14-3-3 protein to YAP causing its cytoplasmic retention and its subsequent ubiquitination and degradation[11,12] Prior to it being demonstrated as a mediator of the hippo pathway, mammalian YAP was identified as a protein that bound the proto-oncogene Yes,[13] and as a transcriptional cofactor for the transcriptional enhanced associate domain (TEAD) family of transcription factors. Specifically, YAP had been found to bind to the carboxyl terminus of all TEAD family members and to promote the sequence-specific DNA binding of TEAD to target promoters.[10] YAP/TEAD activate the promoters leading to the transcription of genes associated with fibrosis and cancers, such as the matricellular proteins CCN2 and CCN1.[14–17] These features led to the conclusion that YAP, TEAD, CCN1 and CCN2 are key downstream effector molecules in the canonical mammalian hippo pathway (Figure 1). It should be pointed out, however, that YAP itself can interact with additional transcription factors, including p73;[10] i.e., although this feature is not a focus of this current review, YAP can act as a transcriptional cofactor outside of the canonical hippo pathway.

Mechanotransduction and extracellular matrix (ECM) stiffness has long been understood to facilitate and prolong fibrogenesis (for reviews summarizing the data leading to this concept, please see[18,19]). This concept combined with the demonstrated link between YAP and the pro-adhesive and profibrotic gene CCN2[20] suggested *a priori* that YAP may be activated in response to increased matrix stiffness and therefore would be sensitive to mechanotransduction in cancers. Indeed, this hypothesis proved to be accurate.[21–23] Specifically, YAP is activated in

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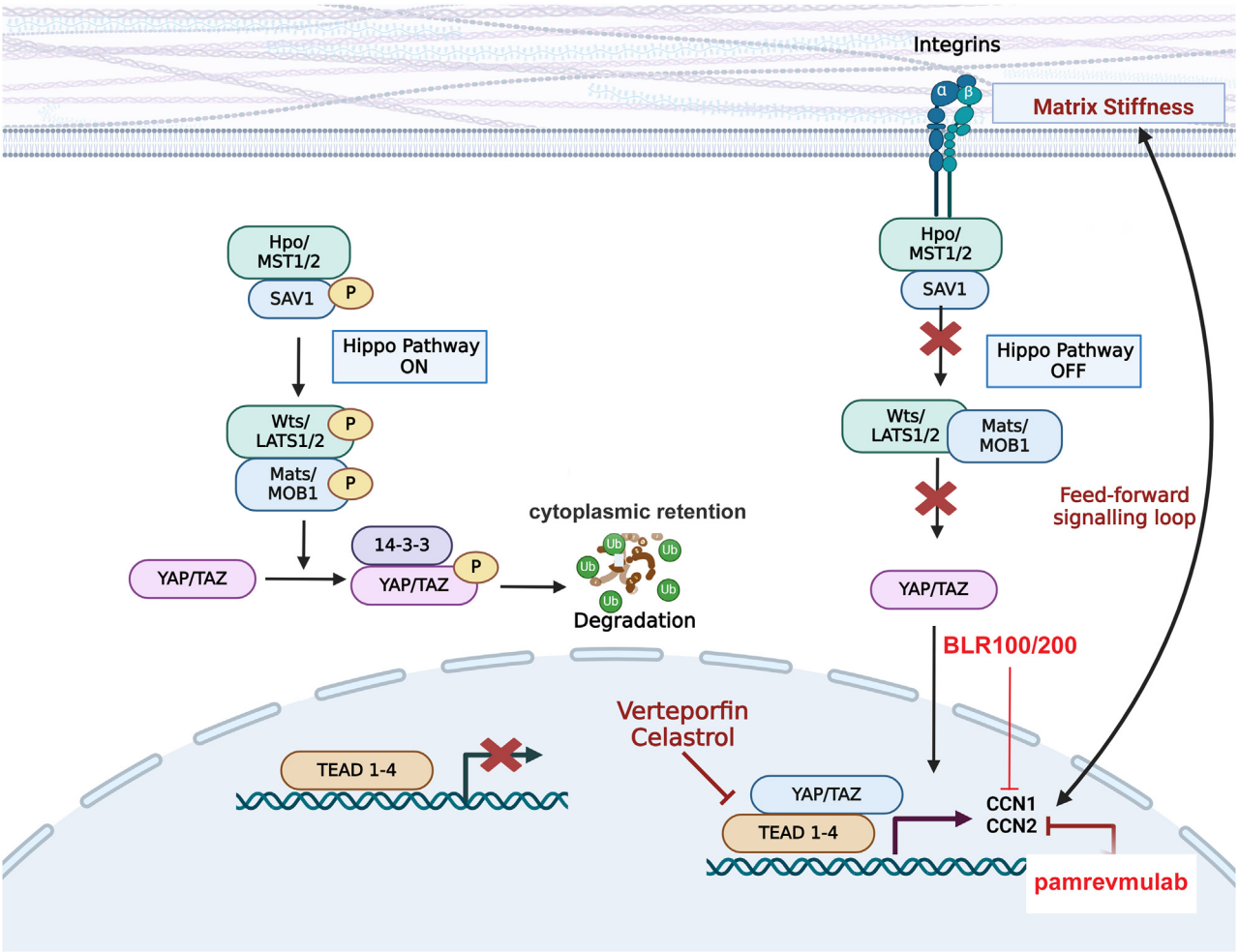

Figure 1. Summary schematic of the canonical mechanosensitive YAP pathway

Note that when the hippo pathway is active (left hand side) YAP is phosphorylated and targeted for degradation. Conversely, when the hippo pathway is overridden (right hand side) in response to increases in ECM stiffness and mechanical tension, YAP and its downstream targets CCN1 and CCN2 act in a feedforward pathway to promote cell adhesion to ECM and therefore drive oncogenesis and fibrosis. For details, see text. The drugs verteporfin and celastrol act by antagonizing the interaction between YAP and TEAD. The CCN3-derived peptides BLR-100 and BLR-200 antagonize CCN1 and CCN2 activity. Red X refers to processed blocked (i.e., transcription of target genes or phosphorylation of YAP).

cancer-associated fibroblasts (CAF)s, and the use of siRNAs directed toward YAP indicated that YAP, in a fashion requiring the actomyosin cytoskeleton, was required for CAFs to promote matrix stiffening/remodeling, cancer cell invasion and angiogenesis.[23] Matrix stiffening was further shown to enhance YAP activation, resulting in a feedforward signaling loop that helps maintain the CAF (myofibroblast) phenotype, resembling the situation observed with fibroblasts in fibrosis.[17,23–25] The ability of mechanotransduction to activate YAP requires an intact actin cytoskeleton, and appears to act by overriding hippo-induced YAP phosphorylation.[26] The exact mechanism underlying this switch remains to be clarified.

The consequence of these observations is that targeting the hippo/YAP pathway in fibrosis and cancers has become a topic of much interest (for representative reviews, please see[27–33]). In this review, we focus on the role of the canonical hippo/YAP pathway in controlling ECM/tumor/melanocyte interactions in melanoma.

THE ROLE OF YAP/HIPPO IN MELANOCYTE BIOLOGY

In skin, melanocytes are located on the basement membrane, rich in laminins and collagen type IV, at the dermal/epidermal junction. Skin melanocytes produce and secrete melanin, in melanosomes, which translocate into keratinocytes and cover their nuclei, thereby providing protection against ultraviolet light (UV)-induced damage.[34]

In development, melanocytes originate from the neural crest, a multipotent cell population which will specify multiple cells and tissues, including craniofacial cartilage and bones, melanocytes, and the peripheral nervous system (for reviews see[35,36]). The neural crest generates

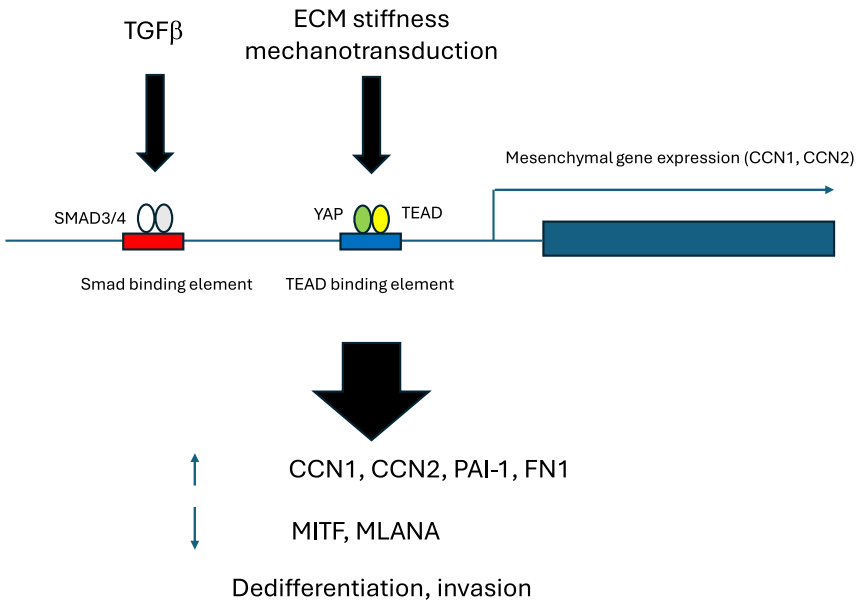

Figure 2. TGFβ/SMAD and YAP/TAZ cooperate to induce a mesenchymal gene expression program in melanoma cells

TGFβ and mechanotransduction, through the canonical Smad and YAP/TEAD pathways, respectively, activate expression of a mesenchymal gene expression program (involving the induction of CCN1, CCN2, plasminogen activator inhibitor protein-1 (PAI-1) and fibronectin-1 (FN-1)), yet suppress differentiation markers such as MITF and melanoma antigen recognized by T-cells 1/melan-a (MLANA).

these numerous cell and tissue types in a process called delamination (division into multiple tissue populations), which involves cell migration from the neural tube (for reviews see[35,36]). In the mouse embryo, the binding to promoters of YAP/Taz and paired box 3 (Pax3), in a TEAD-independent fashion, is essential for expression of genes that promote melanocyte differentiation.[37] Specifically, YAP and Taz are induced in a subset of neural crest cells and activate the expression of the Pax3 target gene microphthalmia-associated transcription factor (Mitf), also known as melanocyte-inducing transcription factor, a gene essential for melanogenesis.[38] This process is impeded by Mst1 and Lats2.[37] YAP also activates the wnt pathway; this activation is required for YAP to induce the melanocyte lineage.[37] Specifically, the *Mitf* promoter is activated by wnt/β-catenin binding to its LEF1/TEF binding element.[39]

Melanocytes are normally in a quiescent state but can be activated in response to cell loss or injury. Quiescent epidermal melanocytes are polarized and bind to the basement membrane through the laminin-binding integrins α3β1 and α6β1, and to keratinocytes through dendrites involving the matricellular protein CCN3 and the collagen IV receptor discoidin domain receptor 1.[40,41] Under these conditions, it has been hypothesized by Kim and colleagues that, in the resting state, melanocytes are not under mechanical stress, and therefore hippo/LATS1/2 are activated.[42] Kim and colleagues further propose that, as with connective tissue fibroblasts, cell loss or injury results in loss of melanocyte contacts with the basement membrane and epithelial cells, resulting in increased mechanical load, thereby resulting in inhibition of LATS1/2 and activation of YAP/TAZ, resulting in increased proliferation and suppression of apoptosis.[42] Although intriguing, this hypothesis has yet to be tested experimentally. A similar situation was suggested to operate in melanoma.

MELANOMA IS STIMULATED BY A PHENOTYPIC SWITCH CAUSED BY MICROENVIRONMENT-PROMOTED YAP ACTIVATION

Melanoma, which develops from melanocytes, is the most dangerous form of skin cancer. Melanoma may also rarely develop in the mouth, intestines or eye (a form called uveal melanoma).[43,44] Epidemiological evidence supports the notion that the primary cause of melanoma is DNA damage caused by solar UV irradiation and a history of childhood sunburn that result in genetic lesions that produce cancerous cells,[45–47] for reviews, see[48,49].

Although melanocytes have stem cell-like properties (for an extensive discussion, please see Hoek and Goding[50]) melanoma progression appears to be driven via the transformation of melanocytes to cancer cells by a so-called phenotypic switch that involves microenvironmental signals acting on melanoma cells. This model posits that genetic lesions yielding constitutive activation of signal transduction pathways (such as ERK) together with microenvironment-mediated changes (for example, from a fibrotic ECM or hypoxia) results in a continuous switching between a differentiated, "proliferative" phenotype (characterized by high expression of neural crest and melanocyte markers), and a dedifferentiated, "invasive" phenotype characterized by low expression of melanocyte markers and high expression of mesenchymal cell markers.[50–53] This mesenchymal phenotype is correlated with the development of drug resistance.[54–57]

In this regard, *a priori*, given their ability to modulate cell/microenvironment/ECM changes, including epithelial-mesenchymal transition, it would be anticipated that wnt, TGFβ and YAP would be involved in this switching.[58–62] Indeed, recent experiments using human cancer cell

Table 1. Druggable targets in the YAP pathway

Target	Drug	Phase in clinical development
YAP/TEAD		
	verteporfin	Approved for macular degeneration
		Pre-clinical for cancer
	celastrol	Pre-clinical
CCN2		
	pamrevmulab	Phase III for pancreatic cancer
CCN1/2 (using CCN3 mimics)		
	BLR100, BLR200	Pre-clinical

lines suggest that TGFβ/SMAD and YAP/TAZ promote an invasive, whereas canonical Wnt signaling promotes a proliferative, phenotype switch.[63] This invasive phenotype includes increased expression of a YAP/TGFβ signature including elevated CCN1 and CCN2 production and reduced expression of MITF[63] (Figure 2). Thus, the YAP/hippo pathway, in concert with TGFβ production is highly implicated in initiating the invasive melanoma phenotype.

THE HIPPO/YAP PATHWAY IN MELANOMA

Indeed, the YAP/hippo pathway is key coordinator of ECM production and angiogenesis in melanoma (for summaries, see[26–32]). For example, in a key study, the Mauviel group[64] examined Hippo pathway component expression in a panel of human melanoma cell lines and melanocytic lesions. YAP and TAZ were found in both the nuclei and cytoplasm of benign nevi and superficial spreading melanoma. Moreover, siRNA knockdown of either YAP or TAZ in cancer cells dramatically reduced anchorage-independent growth, capacity to invade Matrigel *in vitro*, and ability to form lung metastases in mice following tail-vein injection. Conversely, the authors found that YAP overexpression increased melanoma cell invasiveness. These phenotypic alterations were paralleled by altered expression of the YAP target CCN2.[64] In another study YAP facilitated melanoma cell migration via actin-related protein 2/3 complex subunit (ARPC5).[65] YAP expression also contributed to actin remodeling-dependent proto-oncogene B-Raf (BRAF) inhibitor resistance in BRAF V600E mutant melanoma cells.[34] Indeed, BRAF-inhibitor resistant melanoma cells display nuclear localization of both YAP and TAZ concomitant with persistent upregulation of a YAP gene expression signature, as visualized, for example, by significant upregulation of the YAP targets CCN1 and CCN2.[64] Moreover, YAP, via its TEAD interaction domain, promotes proliferation, migration, invasion and transformation of melanoma cells.[59] Indeed, the metastatic potential correlated with TEAD transcriptional activity.[59] Similarly, expression of constitutively active YAP in zebrafish potently induces melanogenesis.[66]

The degree of YAP staining in primary human melanoma samples positively correlates with reduced patient survival.[67–69] Similarly, in a bioinformatics study, down-regulation of latent-transforming growth factor beta-binding protein 4 (LTBP4) expression was seen in human melanoma tissues and cells, and predicted a poor clinical prognosis.[70] LTBP4 overexpression in melanoma cells enhanced phosphorylation (inactivation) of YAP and inhibited CCN1 and CCN2 expression and reduced invasion, metastasis, and proliferation of melanoma cells.[70] Overexpression of YAP rescued this effect of LTBP4.

A recent and extremely intriguing study found that a subset of melanoma patients possessed a mutated version of YAP that was hyperactive; this was the first indication of such a mutation in human cancers.[67] These mutations are serine to alanine mutations at known targets of the hippo pathway.[67] Similarly, ~83% of uveal melanoma patients possess activating mutations in Gαq family members that ultimately result in the rac/cytoskeleton-dependent stimulation of YAP.[71] These observations indicate that. in some melanoma patients, mutations can result in the activation of YAP.

Collectively, the above observations suggest that antagonizing YAP, or its downstream effectors CCN2 or CCN1, may have therapeutic value in treating melanoma.

YAP INHIBITORS

In this section, the focus will be on inhibitors that directly target YAP by antagonizing the interaction between YAP and TEAD.

Verteporfin

Verteporfin (VP), trade name Visudyne, is a benzoporphyrin derivative clinically used in photodynamic therapy for neovascular macular degeneration.[72] Soon after its initial discovery, VP was found to have anti-adhesive properties in cultured human dermal fibroblasts.[65,73] Further experiments revealed that VP, without light activation, acted as a small molecule inhibitor to impede the association between TEAD and YAP[74,75] (Table 1). Thus, VP, as a YAP inhibitor, has gained interest as a possible anti-cancer drug.[76,77] Indeed, not long after VP was identified, cell culture-based experiments suggested that VP may have therapeutic potential in treating cancers.[78]

Several lines of evidence suggest that VP could be effective in treating melanoma. For example, *in vitro* and *in vivo* experiments indicate that VP, both with and without photodynamic therapy, has effects against melanoma cells.[79–86] For example, *in vitro*, VP inhibited proliferation

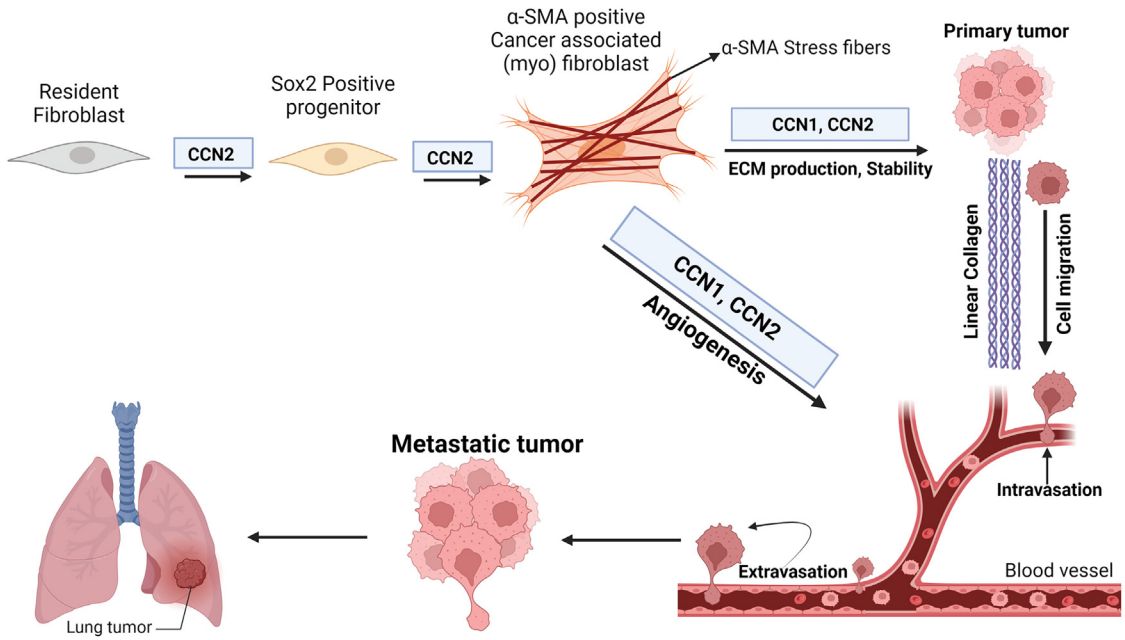

Figure 3. Overlapping and complementary roles of CCN1 and CCN2 in melanoma metastasis

CCN2 expression by universal fibroblasts appears to promote fibroblast plasticity and myofibroblast differentiation; whereas both CCN1 and CCN2 expression by universal fibroblasts appears to promote ECM production and stability. CCN1 and CCN2 are both involved with neovascularization/angiogenesis. For details, see text.

of and induced apoptosis in several uveal melanoma cell lines (specifically 92.1, Mel 270, Omm 1 and Omm 2.3).[85] In addition, VP impaired stem cell properties of these cell lines, including their ability to form melanospheres.[84] Similarly, VP, but not BRAF inhibitors, suppressed the increased ERK1/2 activity and elevated YAP1, TAZ and TEAD expression in BRAF inhibitor-resistant melanoma cancer stem cells.[85] The same study provided an *in vivo* context for their results by using xenografts to show that treating BRAF inhibitor-resistant tumors with VP not only reduced YAP/TAZ expression and restored BRAF inhibitor suppression of ERK1/2 signaling, but also significantly impaired tumor growth.[85] In an a more recent study, VP was shown *in vitro*, using Matrigel and transwell assays, to reduce A375 melanoma cell migration and invasion, accompanied by decreased CCN1 expression.[86] Moreover, the same authors reported that, in uveal melanoma patients, TAZ levels negatively correlated with overall survival.[86]

Additional evidence supporting the concept that VP could be useful in the treatment of melanoma emerged from a recent study examining sirtuin 5 (SIRT5), itself a potential therapeutic target. SIRT5, a predominantly mitochondrial enzyme, promotes metabolic reprogramming, including activation of the electron transport chain and Krebs cycle.[87] A recent report revealed that SIRT5 deacetylated TAZ, causing TAZ nuclear retention culminating in increased binding to the CCN2 promoter and elevated CCN2 expression in A375 cells.[88] The same study reported that SIRT5 overexpression, in a VP-sensitive fashion, significantly increased metastasis of tail vein-injected B16F10 melanocytes to the lung.[88]

Collectively, these studies highlight the potential importance of the YAP/TAZ/CCN axis for treating metastatic melanoma.

Celastrol

Celastrol, a pentacyclic triterpenoid extracted from the roots of traditional Chinese medicine *Tripterygium wilfordii*, was identified, in a high-throughput screen, as a novel inhibitor of the interaction between YAP/TAZ and TEAD[89] (Table 1). Celastrol acts as an antifibrotic agent and exhibits significant broad-spectrum anticancer activities.[90–92] Celastrol blocks proliferation of melanoma cells *in vitro* and *in vivo* and metastasis in mouse models, associated with impairment of adhesive signaling.[93–96] Celastrol also has antitumor activity in C57BL/6 mice bearing B16F10 tumors by both inhibiting tumor growth and increasing $CD8^+$ T cells tumor penetration.[97] The activity of celastrol is also associated with reduced CCN1 and CCN2 expression.[91,98] Although poor water solubility and toxicity have limited the clinical potential of celastrol, much effort is being expended to circumvent these issues; for example, through the development of nanoparticle delivery systems.[99]

THE YAP TARGETS CCN1/CCN2

The CCN family of matricellular proteins have attracted interest due to their roles in modulating fibrosis and cancer progression, for reviews see.[100,101] Including in melanoma cells, CCN1 and CCN2 expression is induced by TGFβ, RAS/MEK/ERK, hypoxia and mechanotransduction; indeed, they are considered prototypical YAP targets.[13,14,102–107] CCN1 and CCN2 are pro-adhesive and signal through a variety of integrin

heterodimers to promote angiogenic and ECM programs *in vivo* and *in vitro*.[108–112] Mice postnatally deleted for either CCN1 or CCN2 in collagen-lineage fibroblasts are resistant to the syngeneic B16F10 model of melanoma metastasis.[113–117] Specifically, loss of CCN1 or CCN2 from fibroblasts expressing a cre recombinase controlled by a promoter/enhancer derived from the human COL1A2 gene, later found to be expressed in so-called universal fibroblasts, resulted in reduced metastasis of subcutaneously injected B16F10 cells to the lung[114,115] This reduced metastasis was accompanied by impaired angiogenesis (including vasculogenic mimicry) and ECM deposition.[114–117] In patients, CAF-specific expression of CCN1 and CCN2 negatively associated with clinical outcome, and positively with checkpoint inhibitor resistance.[114–117] Intriguingly, CCN2 appears to be involved with CAF plasticity, notably of universal fibroblasts, and myofibroblast differentiation,[117] whereas CCN1 appears to be principally involved with ECM stability[114] (Figure 3). Both CCN1 and CCN2 are associated with ECM/microenvironment-induced drug resistance in melanoma, and in other cancers.[114,2,118–120]

Since CCN1 and CCN2 have overlapping and complementary functions *in vivo* (Figure 3), it may be blocking either of these proteins alone, for example with the anti-CCN2 antibody pamrevlumab (aka FG-3019)[121–123] (Table 1), may be insufficient to achieve significant effects in patients. An alternative approach may be to impair their action through the application of an inhibitory protein, CCN3, or small peptides derived from the inhibitory CCN family member CCN3, such as BLR-100 or BLR-200 currently in development. Although these two peptide drugs have not yet been tested in melanoma, they have been shown to be effective in impairing progression in an animal model of human of pancreatic ductal adenocarcinoma (PDAC), where they greatly reduce the formation of the desmoplastic stroma, retard angiogenesis, suppress the formation of ascites (a biomarker of poor prognosis in humans), and the overall growth of the tumor[124] (Table 1). Nonetheless, these findings are consistent with early *in vitro* data showing that YAP expression in CAFs coordinated ECM production and angiogenesis[23] and imply that the functional mediators of YAP action *in vivo* may be CCN1 and CCN2, making them suitable targets for drug intervention. Indeed, it is tempting to speculate that CCN1 and CCN2 may deserve consideration as therapeutic targets for most, if not all, solid tumors.

Conclusion

Since the discovery that the hippo pathway plays a role in cancers by suppressing the proto-oncogene YAP, much interest has been expended on developing drugs that target YAP. Of note, the small molecule inhibitors verteporfin and celastrol have been identified based on their ability to block the interaction of the transcriptional cofactor YAP with its transcription factor target TEAD. Both these molecules appear to be effective in preclinical models of melanoma. Similarly, the downstream YAP targets CCN1 and CCN2, which could be targeted using CCN3-derived peptides, are required for angiogenesis, matrix remodeling and metastasis in a mouse melanoma model. These data strongly suggest that the YAP/CCN axis deserves consideration as a therapeutic target in melanoma.

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AUTHOR CONTRIBUTIONS

A.L. wrote the first draft of the manuscript. P.C. and A.N. designed the figures. J.N., A.N., P.C., and B.L.R. edited the manuscript.

DECLARATION OF INTERESTS

B.L.R. is founder and CEO of BLR Bio and holds patents regarding the development of BLR100 and BLR200 as anti-fibrotic agents.

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