OVOL1/2: Drivers of Epithelial Differentiation in Development, Disease, and Reprogramming

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Abstract

OVOL proteins (OVOL1 and OVOL2), vertebrate homologs of *Drosophila* OVO, are critical regulators of epithelial lineage determination and differentiation during embryonic development in tissues such as kidney, skin, mammary epithelia, and testis. OVOL can inhibit epithelial-mesenchymal transition and/or can promote mesenchymal-epithelial transition. Moreover, they can regulate the stemness of cancer cells, thus playing an important role during cancer cell metastasis. Due to their central role in differentiation and maintenance of epithelial lineage, OVOL overexpression has been shown to be capable of reprogramming fibroblasts to epithelial cells. Here, we review the roles of OVOL-mediated epithelial differentiation across multiple contexts, including embryonic development, cancer progression, and cellular reprogramming.

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Introduction

OVOL1 and OVOL2 are crucial transcription factors of epithelial lineages during vertebrate embryonic development and are involved in the maintenance of an epithelial state and terminal differentiation during tissue homeostasis (Fig. 1) [MacKay et al., 2006; Nair et al., 2006; Teng et al., 2007; Kohn et al., 2014; Sun et al., 2019]. OVOL1/2 can inhibit epithelial-mesenchymal transition (EMT) by directly repressing EMT-inducing transcription factors such as ZEB1, ZEB2, TWIST, and promote the reverse of EMT, mesenchymal-epithelial transition (MET), by inducing the expression of the cell-cell adhesion molecule E-cadherin [Aue et al., 2015; Kitazawa et al., 2019; Watanabe et al., 2019]. Thus, OVOL1/2 can be thought of as one of the MET-inducing transcription factors, similar to GRHL2 [Frisch et al., 2017; Mooney et al., 2017]. However, specific targets and functions of OVOL1 and OVOL2 are relatively poorly understood. A deeper appreciation of how OVOL1 and OVOL2 regulate epithelial differentiation and inhibit EMT will be required in the context of cancer metastasis, which remains the leading cause of cancer-related death worldwide.

Table 1. Chromosomal location and expression pattern of OVOL proteins

OVOL	Chromosomal location		Tissue-specific expression		Spliced variants	References
	mouse	human	mouse	human		
OVOL1	Chromosome 19	11q13	Epidermis, kidney, testis	Placenta, fetal kidney, pancreas	–	Li et al. [2002a]; Chidambaram et al. [1997]; Dai et al. [1998]; Kumar et al. [2012]
OVOL2	Chromosome 2	20p11.2–p12	Testis, skin, stomach, kidney, intestine	Ovary, heart, skeletal muscle	Ovol2A, Ovol2B, Ovol2C	Li et al. [2002a]; Kumar et al. [2012]
OVOL3	Chromosome 7	19q13.1	–	Testis	–	Li et al. [2002a]; Kumar et al. [2012]

Fig. 1. Specific and overlapping functions of OVOL1/2. OVOL proteins are crucial regulators of epithelial differentiation – they can inhibit EMT and induce MET across contexts.

OVOL Proteins

OVO is a nuclear protein expressed specifically in the female germline, and is critical for oogenesis and sex differentiation in *Drosophila melanogaster* [Oliver et al., 1987; Garfinkel et al., 1992; Mével-Ninio et al., 1995; Chidambaram et al., 1997]. *ovo* shares most of its coding sequence with *shavenbaby* (*svb*), which is involved in epidermal morphogenesis [Mével-Ninio et al., 1995; Payre et al., 1999]. *ovo/svb* forms a complex gene locus with separate control regions performing two genetic functions: sexual differentiation and epidermal differentiation [Garfinkel et al., 1992]. OVO/SVB triggers F-actin redistribution that initiates cytoskeleton remodeling, thus functioning as an important regulator of epidermal differentiation [Delon et al., 2003]. The carboxyl terminal containing zinc finger domain (tetrad of C2-H2) of OVO is known to be evolutionary conserved from metazoans to vertebrates. Addition of various non-conserved sequences to primarily the N-terminus region of the gene

gave rise to different OVOL (OVO-like) genes during the course of evolution [Kumar et al., 2012].

Vertebrates have 3 *ovol* genes (*ovol1–3*), among which *ovol1* and *ovol2* are relatively better studied (Table 1). Functional studies in model organisms have shown *ovol* genes to be involved in the development and differentiation of a number of epithelial lineages, such as the skin, kidney, and mammary epithelia [Oliver et al., 1987; Mével-Ninio et al., 1991; Dai et al., 1998; Payre et al., 1999; Johnson et al., 2001; MacKay et al., 2006; Nair et al., 2007]. OVOL1, the first OVOL protein identified in mouse, was shown to be expressed in epidermis (skin, hair follicles, and interfollicular epidermis), kidney, and male germinal epithelia [Dai et al., 1998]. *ovol1*-deficient mouse displayed aberrant hair patterning, cystic kidneys, and defective spermatogenesis [Dai et al., 1998; Li et al., 2005]. OVOL1 has been shown to function downstream of the WNT/ β -catenin/LEF1 pathway in differentiating epidermal cells and hair follicles [Li et al., 2002b], and downstream of the TGF- β /BMP-7/SMAD4 signaling pathway in keratinocytes [Kowanetz et al., 2004]. OVOL1 transcriptionally repressed its target genes such as *c-myc* and *zeb1* by binding to their promoter at a specific promoter sequence – CCGTTA – a recognition sequence that is also present in the *ovol1* promoter, indicating its possible self-inhibition [Tsuji et al., 2018].

Similar to OVOL1, OVOL2 has been shown to be crucial for mouse embryonic development and *ovol2* mutants show embryonic lethality [MacKay et al., 2006; Unezaki et al., 2007]. It is expressed during early-mid embryogenesis, particularly in the inner cell mass and in ectoderm-derived tissues at later stages. OVOL2 expresses abundantly in the testis; however, its expression has also been found in skin, stomach, intestine, ovary, heart, and skeletal muscle [Li et al., 2002a].

Mouse *ovol2* consists of 5 exons, 2 of which are alternatively used to form spliced variants of OVOL2:

OVOL2A (lacking 2nd exon) and OVOL2B (lacking 1st exon) [Li et al., 2002a]. The human *ovol2* gene with 6 exons also has OVOL2A and OVOL2B transcript variants similar to mouse [Li et al., 2002a]. *ovol2* null mutant mice showed expansion of the neuroectoderm causing failure of the closure of the cranial neural tube [MacKay et al., 2006]. Furthermore, defects in embryonic and extraembryonic vascularization along with improper heart development was also observed [Unezaki et al., 2007]. In addition, OVOL2 has been shown to function downstream of BMP signaling during the neural/non-neural cell fate decision in chick embryos [Zhang et al., 2013]. Moreover, *ovol2* is a downstream target of OVOL1: OVOL1 represses transcription of *ovol2* by directly binding to its promoter [Tsuji et al., 2018]. In addition, OVOL1 and OVOL2 recognize nearly identical DNA sequences for binding to their target genes (Table 2), suggesting possible regulation of one another and/or themselves [Wells et al., 2009; Lee et al., 2014].

OVOL in Embryonic Development

OVOL1 has been shown to regulate the proliferation of epidermal cells during embryonic development in vivo using mouse models and in vitro using isolated keratinocytes from mouse [Nair et al., 2006]. OVOL1 was crucial for the terminal differentiation and restricting the proliferative potential of embryonic epidermal progenitor cells. In addition, cultured keratinocytes lacking OVOL1 failed to restrict their growth under growth-inhibitory signals. *ovol1*-deficient suprabasal cells showed upregulated c-MYC, which was shown to be transcriptionally repressed by OVOL1 by directly binding to its promoter, suggesting that OVOL1 is required to repress *c-myc* for restricting cell proliferation [Nair et al., 2006].

OVOL2 has been shown to be capable of inducing mesendodermal fate in mouse development under the influence of BMP signaling – a known regulator of neural/non-neural cell fate decisions. BMP signaling inhibits neural differentiation and promotes the differentiation of mesoderm, endoderm, and epidermis [Zhang et al., 2013]. Thus, OVOL2 was found downregulated during neural differentiation of mouse embryonic stem cells (mESCs). Besides, inhibiting OVOL2 in mESCs facilitated neural differentiation and inhibited mesendodermal differentiation. Moreover, in vivo experiments in chick embryos have shown that BMP4 upregulates OVOL2 through SMAD1/5/8, which directly binds to the second intron of *ovol2* and activates it [Zhang et al., 2013]. Ectopic expres-

sion of OVOL2 in the neural plate represses the expression of SOX2, a definitive marker of the neural plate [Zhang et al., 2013]. Thus, OVOL2 seems to control proper germ layer development in multiple model organisms.

Epidermal/Skin Differentiation

OVOL1 was first characterized functionally in mouse (gene: *movo1*) where it was shown to express in differentiating cells of epidermis and hair follicles, similar to its fly counterpart. It was found to be involved in hair and sperm formation as well – *movo1*^{-/-} mice produce aberrant hair and exhibit a limited ability to reproduce [Dai et al., 1998]. *ovol1* mutant mice show perinatal lethality accompanied with epithelial cysts in the kidney of embryonic onset, and delayed acquisition of the skin barrier [Teng et al., 2007]. Loss of OVOL1 was compensated by OVOL2 which was upregulated in *ovol1*-deficient epidermis. This effect can be mediated by direct repression of *ovol2* by OVOL1 through two OVOL1 recognition sequences in its promoter (CCGTTA) [Teng et al., 2007]. *ovol2*-deficient mice display compromised wound healing characterized by aberrant epidermal cell migration and proliferation and defects in hair follicle matrix cell proliferation and differentiation [Hong et al., 2015].

The reciprocal regulation of epithelial and mesenchymal genes, as seen in EMT/MET, such as a mutually inhibitory feedback loop between OVOL1/2 and ZEB1 [Roca et al., 2013], is also implicated during the differentiation of embryonic ectoderm to neuroectoderm and surface ectoderm [Kitazawa et al., 2016]. Similar reciprocal inhibitory loops are a hallmark of multiple cell-fate decisions during embryonic development and disease [Zhou and Huang, 2011; Sahoo et al., 2020]. While neuroectoderm derivatives expressed various mesenchymal genes, the corneal epithelial cells (CECs) arising from surface ectoderm have a higher expression of epithelial genes such as *cdh1*, *cldn1*, and *krt3*, and downregulated mesenchymal genes such as *zeb1* and *zeb2*. OVOL2 is an important regulator of the human CEC transcriptional program [Kitazawa et al., 2016]. The OVOL1/OVOL2-ZEB1 axis seems to be relevant in epidermal progenitor cells too: *ovol1/ovol2* conditional double knockout mice had blocked terminal differentiation and increased proliferation of embryonic epidermal progenitor cells with defects in α -catenin-driven actin cytoskeleton reorganization and adhesive maturation [Lee et al., 2014]. In addition, *ovol1/ovol2*-deficient epidermal cells displayed increased expression of c-MYC and p63. These are transcription factors involved in stemness which were shown to be

Table 2. Transcriptional targets of OVOL1 and OVOL2 proteins

Target gene	A/R	Model system/patient data	Supporting experiment	Protein	Functional role	References
<i>cdh1, cldn4, rab25</i>	A	Mouse	–	OVOL2	Lumen expansion and barrier formation in renal epithelia	Aue et al. [2015]
<i>vim, zeb1, snai2</i>	R	Mouse	ChIP-Seq	OVOL2A	Mammary morphogenesis and regeneration by inhibition of EMT.	Watanabe et al. [2014]
<i>zeb1</i>	R	SCC-152 cells and patient samples	ChIP	OVOL1	Inhibition of oral SCC growth and metastasis	Xu et al. [2019]
<i>c-myc</i>	R	Mouse	EMSA, ChIP	OVOL1	Growth arrest of embryonic epidermal progenitor cells	Nair et al. [2006]
<i>twist</i>	R	A549 cells, patient samples	ChIP, luciferase reporter gene assay	OVOL2	Inhibition of EMT in lung adenocarcinoma	Wang et al. [2017]
<i>zeb1</i>	R	MG-63 and SW1353 cells, patient samples	ChIP, luciferase reporter gene assay	OVOL2	Induction of MET in osteosarcoma	Liu et al. [2018]
<i>zeb1</i>	R	CNE2 cells and its subclones S18 and S26, tumor samples	ChIP	OVOL2	Fine-tuning of EMT in nasopharyngeal carcinoma	Qi et al. [2018]
<i>c-myc, notch</i>	R	293T, HaCaT, cells	ChIP, luciferase reporter gene assay	OVOL2A	Suppression of cell cycling and terminal differentiation of keratinocytes	Wells et al. [2009]
<i>zeb1</i>	R	A431 cells, cSCC tissue samples	–	OVOL2	Suppression of actinic keratosis to cSCC progression	Murata et al. [2020]
<i>myc, id1, tp63, ascl2</i>	R	BeWo trophoblast cells, HEK-293FT cells	ChIP	OVOL1	Regulation of trophoblast progenitor cell fate during human trophoblast development	Renaud et al. [2015]
<i>c-myc, ovol2</i>	R	A431 cells, cSCC and Bowen's disease skin tissue samples	–	OVOL1	Regulation of shift from Bowen's disease to cSCC	Ito et al. [2017]
<i>id2</i>	R	Mouse, mouse trophoblast stem cells (F4 line), HEK-293T	ChIP, luciferase reporter gene assay	OVOL2	Promotes differentiation of trophoblast stem cells and placental development	Jeyarajah et al. [2020]
<i>p63</i>	R	Mouse keratinocytes	ChIP	OVOL2	Proliferation of epidermal basal cells	Lee et al. [2014]
<i>smad4</i>	R	Mouse	ChIP, EMSA, luciferase assay reporter gene assay	OVOL2	Mammary tumor metastasis	Wu et al. [2017]
A, activation; R, repression; SCC, cutaneous squamous cell carcinoma; cSCC, cutaneous SCC.						

transcriptionally repressed by OVOL2 and exhibited changes similar to EMT which was reversed upon the inhibition of the EMT regulator ZEB1 [Lee et al., 2014]. Contrarily, another study has suggested the transient expansion of HaCaT keratinocytes upon OVOL2 depletion with a loss of long-term proliferation potential and suppression of terminal differentiation [Wells et al., 2009], suggesting a potential context-dependent mediation of the functions of OVOL2.

Deletion of ZEB1 has also been shown to restore the directional migration of bulge hair follicle stem cells (Bulge HSCs) in *ovol2*-deficient mice which display characteristics of reduced proliferation and enhanced EMT [Haensel et al., 2019]. Overexpression of OVOL2 in mice epidermal basal cells disrupts the cytoskeleton structure and displays a defective basal keratin network and defects in their association with hemidesmosomes, adhesion structures which anchor keratin filaments to the cell/basement membrane, resulting in skin blistering [Lee et al., 2017]. Put together, these studies suggest that an epidermal differentiation program mediated by OVOL1 and/or OVOL2 may at least partly be mediated by their inhibition of EMT drivers such as ZEB1.

Mammary Morphogenesis/Renal Morphogenesis

Another developmental scenario where the role of OVOL2 has been investigated is mammary and renal morphogenesis. Reminiscent of observations in epidermal differentiation, OVOL2 has been shown to be required for proper morphogenesis and regeneration of mammary epithelial cells by inhibiting EMT, as revealed through in vivo conditional knockout and lineage-tracing experiments using mouse models. OVOL2 repressed EMT inducers such as ZEB1, ZEB2, and TWIST, and a TGF- β -mediated EMT was observed upon OVOL2 deletion [Watanabe et al., 2014]. Furthermore, OVOL2 deletion blocked mammary ductal morphogenesis, depleted the stem and progenitor cell population, and induced EMT in epithelial cells in an in vivo mouse model. The phenotype associated with OVOL2 depletion was found to be reversible by inhibiting ZEB1 or TGF- β , further strengthening the importance of repressed EMT in ductal morphogenesis [Watanabe et al., 2014].

The GRHL2/OVOL2 pathway is involved in mouse renal epithelial morphogenesis through regulating lumen expansion and barrier formation in collecting duct epithelia by reinforcing the expression of CDH1, CLDN4, and RAB25. GRHL2 transactivated OVOL2 expression by associating with H3 lysine 4 trimethylation, thus functioning upstream of OVOL2. Moreover, OVOL2 induc-

tion was sufficient for the activation of CDH1, CLDN4, and RAB25, suggesting GRHL2 to be dispensable in the presence of OVOL2 [Aue et al., 2015].

Trophoblast Development

Human placental epithelium consists of cytotrophoblast which either proliferates to maintain a sufficient reservoir of mononuclear progenitor cells or can differentiate, which leads to the fusion of differentiated cells with the overlying syncytium forming syncytiotrophoblast [Renaud et al., 2015]. Syncytiotrophoblast forms the principal epithelial barrier between maternal and fetal blood and is important for nutrient, gas, waste, and water exchange between the two blood circulations and synthesizes various hormones for fetal development and the maintenance of pregnancy [Renaud et al., 2015]. OVOL1 has been shown to be robustly expressed in human placenta upon induction of trophoblast differentiation and is crucial for the differentiation of cytotrophoblast to syncytiotrophoblast that remains largely epithelial [Vićovac and Aplin, 1996]. OVOL1 repressed genes involved in maintaining the progenitor cell state of cytotrophoblasts, such as *myc*, *id1*, *tp63*, and *ascl2*, which have an OVOL1 recognition sequence (CCGTTA) within their proximal promoter regions. Deletion of OVOL1 abrogated cytotrophoblast fusion and inhibited genes involved in trophoblast cell fusion and hormonogenesis [Renaud et al., 2015]. Similarly, OVOL2 has been shown to be critical for the development of functional placenta in mice and OVOL2 depletion shows embryonic lethality. Mouse placenta and trophoblast stem cells show higher expression of OVOL2 which is implicated in trophoblast differentiation. OVOL2 deletion inhibits the differentiation of trophoblast stem cells such that they express higher levels of stem cell-related genes such as *eomes*, *esrrb*, and *id2*, and genes involved in differentiation such as *gcm1*, *tpbpa*, *prl3b1*, and *syna* are downregulated. In addition, ectopic expression of OVOL2 results in the precocious differentiation of trophoblast stem cells [Jeyarajah et al., 2020]. Downregulation of OVOL2 has been implicated in EMT induction upon the implantation of non-invasive bovine trophoblast to the uterine endometrial lining. In vitro experiments with cultured bovine trophoblast cells also showed similar induction of EMT only after attachment to the endometrial epithelial layer via the TEAD3/YAP signaling pathway. OVOL2 downregulation was paralleled with an increase in expression of the EMT transcription factors ZEB1 and SNAI2, EMT markers vimentin and N-cadherin, and reduction in the expression of the epithelial marker E-cadherin [Bai et al., 2018]. This study

Fig. 2. The OVOL/ZEB1 axis correlates with EMT/MET across cancer types. **a** Scatter plot of genes correlated using Pearson's correlation with OVOL1 and ZEB1 in the CCLE dataset. Each dot denotes one gene and the x- and y-axis coordinates are correlation coefficient values of that gene with OVOL1 and ZEB1, respectively. The color of the dots represents corresponding *p* values. Green dots are for genes with *p* < 0.05 with OVOL1 and ZEB1. **b** The same scatter plot but for OVOL2 and OVOL1.

highlights the criticality of balance between EMT (inhibited by OVOL2) and MET (promoted by OVOL2) during embryogenesis and development.

OVOL and EMT in Cancer

EMT and MET are believed to be important for successful metastasis of cancer cells to distant locations. While EMT can trigger invasion and tumor cell dissemination, MET can facilitate the conversion of mesenchymal carcinoma cells to their epithelial phenotype at distant sites where they can regain proliferation [Jolly et al., 2017]. OVOL1 and OVOL2 can induce MET in prostate and breast cancer cells characterized by changes in cell morphology and an increased expression of epithelial markers such as E-cadherin and ESRP1 and decreased levels of EMT inducers ZEB1, ZEB2, and SLUG (SNAI2) [Roca et al., 2013]. Importantly, similar to OVOL1/2, ESRP1 and E-cadherin can also form mutually inhibitory loops with ZEB1, thus repressing EMT [Schmalhofer et al., 2009; Jolly et al., 2018b]. In addition, expression of

OVOL1 and OVOL2 in mesenchymal prostate cancer cells decreased their metastatic potential in orthotopic mouse models of prostate cancer [Roca et al., 2013]. Similarly, OVOL2 has been shown to antagonize TGF- β signaling and inhibited EMT during mammary tumor metastasis by repressing SMAD4 expression and interfering in SMAD4 and SMAD2/3 complex formation [Wu et al., 2017]. Thus, the OVOL1/2-ZEB1 axis can form an important axis of regulation of EMT in cancer progression, as observed for the Cancer Cell Line Encyclopedia (CCLE) cohort [Barretina et al., 2012] (Fig. 2).

The OVOL1-OVOL2 axis has also been implicated in the regulation of c-MYC in Bowen's disease and cutaneous squamous skin cancer. Bowen's disease, characterized by atypical keratinocytes, is a non-invasive variant form of squamous cell carcinoma (SCC) restricted to the epidermis. It is characterized by higher expression of OVOL1 while c-MYC is downregulated. On the other hand, SCCs show an opposite trend: OVOL1 is downregulated while c-MYC levels are higher. OVOL2 expression was downregulated in Bowen's disease and showed a varied expression in SCC, diffused in the cytoplasm

with sporadic expression in nuclei. The expression pattern of OVOL1, OVOL2, and c-MYC in these two forms of skin deformities highlight the role of the OVOL1-OVOL2 axis in restricting cell invasiveness by modulating the expression of proto-oncogene c-MYC [Ito et al., 2017]. A similar observation was made in actinic keratosis (AK), which is characterized by intraepidermal lesions, and the progression of AK to cutaneous SCC (cSCC) is rare. AK displays suppressed EMT owing to higher levels of OVOL1/OVOL2 and lower levels of ZEB1 and vimentin, contrary to higher levels of ZEB1 and vimentin in cSCC and lower levels of OVOL1/OVOL2 [Murata et al., 2020].

Furthermore, in A431 SCC cells, OVOL1 and OVOL2 knockdown increased the mRNA and protein levels of ZEB1. Also, OVOL2 knockdown increased the invasive capability of cells, suggesting OVOL2/ZEB1 crosstalk in modulating AK/cSCC progression [Murata et al., 2020]. OVOL2-mediated suppression of ZEB1 and promotion of MET is also reported in osteosarcoma [Liu et al., 2018]. A recent study has shown that OVOL1 mediated the suppression of proliferation, invasion, and migration in oral SCC (SCC-152) cells by inhibiting ZEB1 expression by directly binding to its promoter [Xu et al., 2019], further endorsing that the OVOL2/ZEB1 feedback loop controls epithelial-mesenchymal plasticity across carcinomas.

Mathematical modeling of the feedback loop between ZEB1 and OVOL2 has revealed that in addition to epithelial (high OVOL2, low ZEB1) and mesenchymal (low OVOL2, high ZEB1), cells can acquire one or more hybrid epithelial/mesenchymal (medium OVOL2, medium ZEB1) states [Hong et al., 2015; Jia et al., 2015; Jolly et al., 2015], which can be the most plastic and aggressive state [Jolly et al., 2018a]. In H1975 lung cancer cells that can display a hybrid E/M stably over multiple passages, knockdown of OVOL2 increased the levels of mesenchymal markers, impaired collective cell migration, and drove a complete EMT [Jolly et al., 2016], reminiscent of its role reported in mammary morphogenesis. In collective migration of terminal end buds during mammary development, and that of lung cancer cell lines, OVOL2 can be thought of as a “phenotypic stability factor” [Jolly et al., 2016] that can prevent “cells that have gained partial plasticity from crossing the line to undergo complete EMT” [Watanabe et al., 2014], thus acting as a gatekeeper of the epithelial phenotype.

Similarly, in A549 cells, OVOL2 can suppress EMT by inhibiting the transcriptional activity of TWIST [Wang et al., 2017]. Low OVOL1 and high ZEB1 and FN1 expres-

sion was also seen in tumor buds of oral SCCs which form the invasive front and are known to display EMT features [Jensen et al., 2015]. Low OVOL2 expression has been shown to be associated with worse overall survival in hepatocellular and colon carcinoma patients and correlated with EMT progression in patient HCC tissue samples [Fu et al., 2016; Ye et al., 2016]. However, a functional connection of OVOL with MET in vivo remains to be identified.

OVOL, Plasticity, and Stemness

Hybrid E/M cells can facilitate collective cell migration, as observed in clusters of circulating tumor cells (CTCs). Relative to individually migrating CTCs, CTC clusters tend to have an increased survival of cancer cells in blood circulation [Saxena et al., 2020]. Moreover, in CTC clusters, the binding sites for stemness-associated factors such as OCT4, NANOG, and SOX2 are more hypomethylated [Gkoutela et al., 2019]. Together, these factors may contribute to the disproportionately high metastatic propensity of CTC clusters [Aceto et al., 2014].

A mechanism-based mathematical model coupling OVOL with EMT (miR-200/ZEB) and the stemness (LIN28/let-7) circuit predicted that OVOL can not only stabilize a hybrid E/M phenotype, but also increase the stemness associated with hybrid E/M and/or epithelial phenotypes, while decreasing the stemness associated with the mesenchymal state. Thus, OVOL can fine-tune the positioning of the “stemness window” on the “EMT axis” [Jolly et al., 2015]. Similarly, an increase in stemness by OVOL2 has been shown in nasopharyngeal cancer, where OVOL2 ectopic expression in mesenchymal cells could only induce a partial epithelial character. In addition, inhibition of ZEB1 in *ovol2*-deficient cells decreased stemness without affecting their invasiveness, suggesting a crucial role of OVOL2 in stabilizing the hybrid E/M phenotype and conferring stemness to it [Qi et al., 2018]. The transient receptor potential vanilloid 1 (TRPV1), a non-selective cation channel, has been shown to modulate the plasticity of hepatocyte. TRPV1 downregulation has been shown to be associated with activation of ZEB1 and inhibition of OVOL2, which promoted hepatocarcinogenesis in a SOX10-dependent manner [Xie et al., 2019]. Coordinated regulation of the cell state by the ZEB1-OVOL2-GRHL2 axis has been shown to be crucial for corneal endothelial cells and alteration in this axis results in posterior polymorphous corneal dystrophy char-

Fig. 3. The OVOL/ZEB1 axis regulating EMT/MET in various biological contexts. OVOL and ZEB1 drive opposite (epithelial vs. mesenchymal, respectively) differentiation in both normal epithelial differentiation as well as in cancer metastasis.

acterized by aberrant activation of the Wnt pathway, highlighting the role of the ZEB1-OVOL2-GRHL2 axis in mediating cellular plasticity in corneal tissue homeostasis [Chung et al., 2019].

OVOL and Cellular Reprogramming

OVOLs are known to be critical regulators of epidermal fate determination and differentiation, indicating their capability to confer epidermal phenotype to other cell types by reprogramming their gene expression [Kagawa et al., 2019]. OVOL1 in combination with some or all of the selected critical regulators of epidermal fate (PRDM1, p63, KLF4, ZNF750, and GRHL3) has been shown to be sufficient for the rapid conversion of human dermal fibroblasts to an induced keratinocyte phenotype expressing keratinocyte-specific genes: *krt14* and *gjb2* [Chen et al., 2014]. Furthermore, OVOL2 has been shown to cooperatively promote MET in fibroblasts to keratinocytes induced by epithelial lineage-promoting transcription factors such as HNF1A, TP63, and KLF4 by inducing genes involved in the epithelial phenotype and repressing fibroblast-associated genes [Watanabe et al., 2019]. Similarly, reprogramming of human fibroblast cells to CECs was achieved by overexpressing the core CEC network consisting of PAX6, OVOL2, and KLF4. In addition,

OVOL2 was sufficient to directly reprogram neural ectoderm to a more epithelial cell state – surface ectoderm [Kitazawa et al., 2019].

Conclusion

OVOL1/OVOL2 are critical regulators of normal epithelial differentiation as well as cancer cell differentiation (Fig. 3). They function downstream of growth signaling such as Wnt/ β -catenin and BMP-SMAD. They can inhibit EMT and promote MET in cancer, and have thus been implicated as prognostic markers for patient survival. OVOL1 and OVOL2 regulate many common targets, perform many redundant functions, and can compensate for each other; however, few targets and functions are specific to OVOL1 and OVOL2. Looking at the diverse roles of OVOL during embryonic development, cancer, and reprogramming, it is important to identify and characterize upstream and downstream targets of OVOL and understand how various OVOL-mediated pathways converge to perform similar functions.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

K.S. prepared a first draft of the manuscript. K.S., S.S., T.C.-T., and M.K.J. prepared the figures and edited the manuscript. M.K.J. supervised the study.

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