AJUBA: A regulator of epidermal homeostasis and cancer

Krista Schleicher¹,² | Daniel Schramek¹,²

¹Molecular, Structural and Systems Biology, Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada
²Faculty of Medicine, Molecular Genetics, University of Toronto, Toronto, ON, Canada

Correspondence
Daniel Schramek, Lunenfeld-Tanenbaum Research Institute, 600 University Ave, Rm. 1081, Toronto, ON, MSG 1X5, Canada.
Email: schramek@lunenfeld.ca

Funding information
Ontario Research Fund Research Excellence Round 8, Grant/Award Number: RE08-065

Abstract
The epidermis, outermost layer of the skin, is constantly renewing itself through proliferative and differentiation processes. These processes are vital to maintain proper epidermal integrity during skin development and homeostasis and for preventing skin diseases and cancers. The biological mechanisms that permit this balancing act are vast, where individual pathway regulators are known, but the exact regulatory control and cross-talk between simultaneously turning one biological pathway on and an opposing one off remain elusive. This review explores the diverse roles the scaffolding protein AJUBA plays during epidermal homeostasis and cancer. Initially identified for its role in promoting meiotic progression in oocytes through Grb2 and MAP kinase activity, AJUBA also maintains cytoskeletal tension permitting epidermal tissue development and responds to retinoic acid committing cells to initiate development of surface epidermal layer. AJUBA regulates proliferation of skin stem cells through Hippo and Wnt signalling and encourages mitotic commitment through Aurora-A, Aurora-B and CDK1. In addition, AJUBA also induces epidermal differentiation to maintain appropriate epidermal thickness and barrier function by activating Notch signalling and stabilizing catenins and actin during cellular remodelling. AJUBA also plays an imperative context-dependent tumor-promoting and tumor-suppressive role within epithelial cancers. AJUBA’s abundant roles within the epidermis signify its importance as a molecular switchboard, vetting multiple signalling pathways to control epidermal biology.

KEYWORDS
Cancer, epidermal differentiation, regeneration, squamous cell carcinoma, stem cells

1 | INTRODUCTION

The epidermis is the outermost layer of the skin and is comprised of a basal and suprabasal layers.¹,² The entire epidermis renews every 2 weeks, and epidermal stem cells play a critical role in maintaining tissue homeostasis by ensuring replacement of cells that are lost during tissue turnover or following wounding.² Differentiation is an integral process of renewal, whereby cells transition through four keratinocyte layers: basal, spinous, granular and cornified – from stem-like to most differentiated cellular state.³ Onset of differentiation is triggered by basal cell detachment from the underlying basement membrane, followed by intensive cell remodelling and stop of proliferation, culminating in terminal cell differentiation into highly cross-linked epidermal scales that are exfoliated from the surface of the skin.⁴ While functionally mimicking human skin, murine skin has several structural differences such as epidermal and dermal thickness and hair follicle density.⁵⁻⁷ Maintaining the epidermal primary function as a barrier is of uttermost importance to protect against mechanical impacts and pressure, variations in temperature, micro-organisms, radiation...
and chemicals and to prevent dehydration. As such, maintaining a properly functioning stem cell pool while at the same time ensuring highly balanced differentiation is regulated by a plethora of signalling pathways and cellular mechanisms. For example, Notch signalling plays a critical role in inducing epidermal differentiation and Notch pathway components are differentially expressed within the different keratinocyte layers.\(^6\) It is well-documented that basal keratinocytes express Delta1(Dll1), while Jagged1 and 2 are expressed in suprabasal cell layers within murine epidermis.\(^1,9\) Jagged1 and 2 are critical NOTCH receptor ligands, stimulating differentiation and stratification of suprabasal keratinocytes, where most studies find expression of NOTCH receptors.\(^1,9-12\) On the other hand, Dll1 participates in cis-inhibition of Notch signalling preventing differentiation in the basal layer.\(^9,13-15\) Thus, NOTCH ligands orchestrate cellular differentiation of the spinous cells by binding to NOTCH receptors, which triggers NOTCH cleavage and release of the NOTCH intracellular domain (NICD), which translocates to the nucleus and binds the CSL/RBPJ transcription factor complex to activate NOTCH target gene expression required for keratinocyte differentiation.\(^11\) The preferential expression of NOTCH pathway proteins has recently been validated via single cell RNA sequencing (online interface for scRNAseq within stratified murine epidermis: https://kasperlab.org/mouseskin).\(^9\)

Regeneration and controlled proliferation of epidermal stem cells within the basal layer is required to consistently renew the epidermis. Regeneration is governed by several signalling pathways, with Hippo signalling taking a central stage. Localization of YAP/TAZ, the transcriptional co-activators of the Hippo signalling pathway, is regulated by LATS1/2 kinase-induced phosphorylation of YAP/TAZ.\(^4,16-19\) LATS-induced phosphorylation inhibits the transcription factors by triggering their cytoplasmic retention and prohibiting nuclear translocation. In addition, YAP/TAZ are also regulated by expression patterns of \(\alpha\)-catenin, adherens junctions and actin, respectively.\(^4,16-19\) Cytoskeletal components relay extracellular matrix stiffness and cell-cell interactions and regulate YAP/TAZ nuclear translocation by several mechanisms such as binding and sequestering Hippo components to cell-cell or cell-matrix attachment sites, actin-stress induced JNK/c-Jun activation to promote LIMD1-LATS1 binding or direct inhibitory phosphorylation of LATS1 by Src in response to integrin- and FAK-activation.\(^20-24\) The core Hippo cascade proteins are expressed throughout the keratinocyte layers. Within the single layer of basal keratinocytes, YAP is almost exclusively localized within the nucleus indicative of proliferating cells,\(^19\) while nuclear accumulation of YAP decreases with increasing differentiation with YAP being exclusively cytoplasmic in granular layers.\(^19\) Nuclear localization of YAP triggers expression of Hippo target genes, promoting both symmetric (=self-renewal) and asymmetric cell division of basal keratinocytes.\(^25-27\) As such, Hippo and Notch signalling appear to have opposing functions – the YIN and YANG of skin biology.

Overall, having a balance of basal cell proliferation and suprabasal cell stratification is not only important for normal function of the skin as protective barrier, but also to prevent tumor initiation. This review summarizes AJUBA as a key gene linking the multitude of signalling pathways such as Hippo and Notch signalling within this balancing act. Since its discovery, AJUBA was shown to function as a scaffolding protein participating in the assembly of a multitude of signalling pathways. As such, it acts as a signalling hub assembling diverse signalling pathways, strategically regulating selectivity and forming cross-talks between signalling pathways. While AJUBA’s precise role in signalling cascades has been recently reviewed elsewhere,\(^28\) we here concentrate on AJUBA’s physiological role during epidermal stem cell biology and carcinogenesis.

### 2 | AJUBA’S DISCOVERY

AJUBA ("curiosity" in Urdu, an Indian dialect) was first discovered in a study looking for interactors of erythropoietin receptor (EPO-R) using yeast two-hybrid screening.\(^29\) This study identified the first functional role of AJUBA, demonstrating that it associates with Grb2 to enhance MAP kinase signalling and that it correlates with meiotic progression within mature Xenopus oocytes.\(^29\) AJUBA, is a 58 kDa protein and belongs to the group 3 LIM-domain containing proteins alongside zyxin, LPP, Trip6, and LIMD1. Group 3 proteins contain 3–4 LIM domains at the C terminus that are rich in cysteine and histidine residues and are known to mediate protein-protein interaction (PPI) as well as distinct N-terminal domains (preLIM domain).\(^29\) Specifically, AJUBA contains 3 tandem C-terminal LIM-domains. LIM-domains are relatively promiscuous in the sense that they tend to physically interact with a large number of proteins such as enzymes, receptors, cytoskeletal proteins thereby participating in several signalling pathways, making it difficult to mechanistically unravel their function.\(^29\) AJUBA’s preLIM domain is abundant in gly-cine and proline residues, a unique feature distinguishing AJUBA from other group 3 members. Specifically, the N-terminus contains two stretches of proline-rich SH3 recognition motifs, another PPI domain, and a nuclear export signal.\(^29\) Within mammals, AJUBA LIM family proteins include the related AJUBA (JUB), LIMD1, and WTIP. Drosophila has a single homolog, djub, with greatest sequence similarity to mammalian AJUBA.\(^30\) These homologs are tightly conserved. AJUBA resides on chromosome 14 in humans and mice and on the X chromosome in drosophila.

### 3 | AJUBA’S ROLE DURING DEVELOPMENT

In development, AJUBA is present in all embryonic germ layers and within foetal components of the developing placenta.\(^31\) Expression of AJUBA dramatically decreases at post-embryonic day 12.5 being limited to skin epidermis, oral mucosa, nervous system and the genitourinary tract.\(^31\) AJUBA-deficient mice are viable with no phenotypic effect on a mixed C57BL/6 J-129 X1/SvJ background.\(^32\)
However, AJUBA-deficiency C57BL/6 J mice are embryonic lethal, indicating that murine background influences the phenotype of AJUBA loss. Similarly, AJUBA-deficiency Drosophila melanogaster flies are embryonic lethal.

During early murine development, cross-talk between dermis and epidermis lead to development of the basement membrane. The ectoderm undergoes FGF signalling by BMP activated of SMAD pathway to commit the ectoderm to epidermal fate, differentiating into keratin-expressing cells, forming the basal layer of the epidermis. During mid-gestation, typically at embryonic day (E) 9.5 in a mouse embryo, the epidermis forms as a single-layer surface ectoderm (Figure 1A). A periderm develops in early stratification to protect the single layer from constant exposure to the amniotic fluid during E10-E12 (Figure 1A). Murine stratification is developed through asymmetric division ending with terminally differentiated, cornified cells; a phenomenon not prominent in human epidermis. AJUBA has been shown to be involved in several signalling cascades and aspects of epidermal development (Figure 1A).

3.1 | AJUBA communicates cytoskeletal tension into a Hippo Signalling response during development

During embryogenesis and tissue morphogenesis, mechanical forces as well as extrinsic mechanical stress induce changes of cell shape, size, position and gene expression. During these dynamic processes, adhesive complexes between cells are constantly resolve and establish anew. Adherens junctions are one of the main molecular complexes responsible for cell-cell recognition and interaction, force transmission, force sensing and force generation. Adherens junctions are composed of cadherins, which are transmembrane proteins that operate as homo- or heterophilic cell-cell adhesion receptors and link to the actin cytoskeleton through p120-, α- and β-catenins. Adherens junctions have several cadherin-catenin clusters regulating cell-cell intercalation and invagination during tissue remodelling. These processes determine cellular fate, define embryonic compartments, and initiate epithelial development. During tissue morphogenesis, adhesion junctions have been reported to interact with microtubules in Drosophila (whereby microtubules are reported
to strengthen these cell-cell junctions specifically by regulating myosin II and β-catenin, myosin and several regulatory proteins to diversify cellular outcomes. The Drosophila AJUBA homolog, Jub, plays a crucial role in the developing embryo by interacting with adherens junctions (and complexed proteins) to regulate epithelial closure and wing development (Figure 1B).

During Drosophila development, Jub is recruited to adherens junctions in response to high actin-myosin contractility. This recruitment regulates the Hippo pathway, for example during the morphogenesis of the Drosophila wing, a tissue composed of epidermal cells. Wing development is dependent on the phosphorylation of Myosin II by Rho-associated kinase and the increased Myosin activity. This recruitment regulates the Hippo pathway, for example during the morphogenesis of the Drosophila wing, a tissue composed of epidermal cells. Wing development is dependent on the phosphorylation of Myosin II by Rho-associated kinase and the increased Myosin activity.

AJUBA has also been shown to be regulated by Retinoic acid (RA) and to mediate RA-induced differentiation (Figure 1C). RA functions as a ligand for the retinoic acid receptor (RAR) and retinoid X receptors (RXR), which upon RA binding heterodimerize in the nucleus, altering the transcriptional activity of these transcription factors from repressors to activators of transcription. RA signalling is conserved throughout development and dictates cellular fate by spatiotemporal distribution, generating an RA gradient within the developing embryo. Cyp26 expression, FGF, and RDH10/RALDH2 control anterior and posterior RA concentration differences. Epidermal development is tightly regulated by RA concentration. For example, high RA amounts promote differentiation of ectodermal cells into epidermal keratinocytes and induces proliferation of basal keratinocytes during embryonic development. Studies on human embryonic stem cells (hESCs) have shown that RA promotes ectodermal cells to gain epithelial properties, expressing the basal keratinocyte marker, keratin 14 (K14). In addition, a decrease in RA leads to terminal differentiation resulting in a stratified epidermis, which happens around E15.5 in mice. Thus, RA commits cells to an epithelial fate by inducing RXR-mediated proliferation and decreases epithelial differentiation.

Using P19 murine embryonal carcinoma cells as an ectodermal model system, Kanungo et al. have shown that full-length as well as the pre-LIM-domain of AJUBA localize to the cytoplasm, while LIM-domain-only localized to the nucleus. Interestingly, RA treatment resulted in nuclear accumulation of full-length AJUBA concomitant with differentiation of the P19 cells and stimulation of c-Jun N-terminal kinase (JNK) activity, known to govern epidermal morphogenesis (Figure 1C). In addition, overexpression of full-length and the pre-LIM-domain of AJUBA led to hyperproliferation, while expression of the LIM-domain only resulted in suppression of proliferation and induced endodermal differentiation. Together, these data show that AJUBA localization is regulated by RA signalling, that nuclear accumulation of AJUBA correlates with induction of differentiation and that expression of truncated and constitutive nuclear AJUBA induces differentiation, indicating that AJUBA is tightly involved in maintaining proliferative capacity and could suggest a role in ectoderm identity. Opposed to early cell fate commitment, in adult epithelium, RA is known to inhibit keratinocyte differentiation, but AJUBA has not been linked to RA signalling pathway in the adult epithelium.

These data show that AJUBA is an integral part of several cell-cell and cell-matrix complexes and regulates several cellular phenotypes such as adhesion, proliferation, differentiation and migration.

## 3.2 Retinoic Acid promotes nuclear accumulation of AJUBA during ectoderm differentiation

AJUBA’s best characterized role is its negative regulation of the Hippo signalling pathway (Figure 2A). The Hippo pathway is highly conserved and best known for regulating tissue and organ size. Hippo signalling is also required in many other processes, such as epithelial to mesenchymal transition and cellular polarity. The Hippo pathway was initially characterized in Drosophila utilizing larval wing and eye imaginal disc epithelium. The core of this pathway consists of a kinase cascade involving Hippo [Hpo; Drosophila homolog of mammalian Ste20-like kinases (MST) 1/2] and Warts [Wts; Drosophila homolog of mammalian large tumor suppressor (LATS) 1/2], which transmits plethora of intra- and extracellular signals to control phosphorylation of the transcription co-activator
Yorkie (Yki; *Drosophila* homolog of mammalian YAP/TAZ), thereby restricting its activation and nuclear translocation. *Jub* (*Drosophila* homolog of mammalian AJUBA) is downstream of hippo and interacts with Wts and Sav to inhibit Yki phosphorylation, thus permitting nuclear accumulation of Yki, depicted in Figure 2A.67 Flies hemizygous for the *Jub* locus die in the late embryonic to first instar larva stage.61 Mammalian AJUBA can rescue growth impediment when *Jub* is knocked down in *Drosophila*, signifying AJUBA's conserved function across species.67 In the stem cell-enriched, proliferating, basal keratinocyte layer of mammalian epidermis, Hippo signalling is shut down and YAP is in the nucleus, while non-proliferating suprabasal cells exhibit active Hippo signalling (=Yap is cytoplasmic). Analogous to *Drosophila*, AJUBA was shown to bind the mammalian orthologues, LATS1/2 (Wts) and WW45 (Sav) through co-immunoprecipitation experiments in human epidermal cells.67 Via sequestration of LATS, AJUBA thus functions as a negative regulator of the Hippo pathway, allowing nuclear translocation of YAP/TAZ. The interaction of AJUBA-LATS1/2 is regulated by cellular tension and cell crowding, where decreased cellular contact leads to increase in AJUBA-LATS1/2 interaction and increased nuclear YAP.67 AJUBA acts as a switchboard, integrating multiple signalling pathways to regulate the activity of YAP. For example, EGFR signalling regulates proliferation within the basal cell layer of the epidermis by promoting nuclear localization of YAP.68 In *Drosophila*, Ras-MAPK signalling leads to phosphorylation of *Jub* through direct ERK-dependent phosphorylation, which leads to increased binding of *Jub* to Wts and Sav resulting in increased Yki nuclear translocation, demonstrating a negative regulation of the Hippo pathway governed by AJUBA (Figure 2A).68 This signalling relay is conserved in mammals and EGFR-RAS-MAPK signalling promotes phosphorylation of the AJUBA, which is required for EGFR activation of the Hippo pathway.68 Similarly, JNK increased the binding between AJUBA-family proteins to LATS1/2 through direct JNK-dependent phosphorylation.69 JNK signalling maintains epithelial homeostasis by inducing regeneration of the basal cell layer during stress and repair.69 In addition, JNK signalling induces Yki to translocate to the nucleus, which is blocked by depletion of *Jub*.69 These data show that cellular tension and cell crowding as well as MAPKs such as ERK and JNK regulate AJUBA's localization or phosphorylation to inhibit the Hippo pathway and thus stimulate the transcriptional activation of genes that promote proliferation.

4.1.2 | AJUBA promotes kinase-dependent Mitotic Commitment

AJUBA was identified as a binding partner and important for the activation of Aurora-A (Figure 2B). Aurora-A kinase promotes entry into mitosis, centrosome maturation, and the formation of spindles.70 Aurora-A contributes to centrosome separation only after nuclear envelope break-down and is important for maintaining and separating the centrosomes.71 Aurora-A deficient mice have severe deficiencies in the number of basal keratinocytes within the epidermis at E13.5 and die shortly after birth.72 Centrosome abnormalities are abundant within these basal keratinocytes, leading to division and stratification impairments.72 AJUBA was shown to interact with Aurora-A during mitosis and to facilitate autophosphorylation of Aurora-A. Cells synchronized at G2-M demonstrated increased AJUBA-centrosome localization and AJUBA knockout cells exhibited impaired activation of Aurora-A and subsequent mitotic entry.71 Both AJUBA PreLIM and LIM domain bind to Aurora-A’s C- and N-terminus, respectively (Figure 2B).73 In 293 T cells, the binding of AJUBA to Aurora-A prevents auto-inhibition of Aurora-A, thus AJUBA binding is required for phosphorylation of Aurora-A and mitotic commitment (Figure 2B).74 Corroborating these
findings, in Drosophila neuroblasts, Ajuba was shown to localize at mitotic poles with Aurora-A.75

While AJUBA was clearly shown to be involved in mitotic checkpoint, chromosome dynamics, and cytokinesis within epithelial cells, this was not yet explored specifically within the epidermis.70 In HeLa and COS-1 cells, AJUBA also forms a complex with Aurora-B and BUBR1 kinases at kinetochores.75 Specifically, AJUBA binds via its PreLIM domain to microtubules and kinetochores during metaphase and anaphase respectively, aiding in metaphase to anaphase transition.75

In addition, AJUBA was shown to be regulated by the cyclin-dependent kinase CDK1. CDK1 bound to cyclin B governs cellular mitosis, but CDK1 can compensate for other CDK proteins by binding with all cyclins.76-78 AJUBA was found to be phosphorylated and regulated by CDK1 during prometaphase/metaphase of mitosis.79 Interestingly, binding of AJUBA with LATS1 occurs regardless of the phosphorylation status of AJUBA and thus Hippo pathway activation seems to be independent of AJUBA phosphorylation status. This also suggests that mitotic phosphorylation of AJUBA controls cell cycle independent of Hippo, albeit one has to mention that AJUBA itself had no effect on Hippo pathway activation in the examined cells (HPNE and RCA) and might thus be context-specific.79 Mitotic phosphorylation of AJUBA is essential for AJUBA to promote cellular proliferation, anchorage independent growth and tumor growth. Together, these data establish AJUBA as an oncogene promoting cell survival by regulating cell cycle. While this phenomenon has yet to be explored in keratinocytes (murine nor human), these data show that AJUBA is tightly involved in cell cycle control especially during various aspects of mitosis.

4.1.3 | Wnt signalling

Canonical Wnt signalling involves Wnt-ligands binding to Frizzled receptors and Wnt-co-receptors such as LRPS/6 to initiate a signal cascade that result in accumulation of β-catenin and its nuclear translocation, where β-catenin acts as co-activator of TCF/LEF family transcription factors (Figure 2C).43 Non-canonical WNT signalling is independent β-catenin, involves different co-receptors such as ROR, PTK7 or RYK and regulates planar cell polarity (via RhoA and JNK pathways) or activates calcium signalling through calcium release from the endoplasmic reticulum (ER) (via PKC and NFAT).43 Wnt signalling in the epidermis is regulated via cross-talk with several other signalling pathways such as Notch or Hippo signalling. Proliferating basal keratinocytes exhibit nuclear β-catenin indicative of active WNT signalling, while NOTCH1 is expressed within the stratified suprabasal cells, where NOTCH1 actively represses β-catenin signalling to limit Wnt-β-catenin-dependent proliferation.80 Furthermore, YAP/TAZ are essential proteins for the degradation of β-catenin.43,81 YAP/TAZ binds directly to phosphorylated β-catenin acting like a chaperone for simultaneous degradation of both proteins by β-TrCP complex.81 Upon activation of WNT signalling cascade, YAP/TAZ are released from the destruction complex leading to simultaneous increase of β-catenin and YAP/TAZ and concomitant nuclear accumulation, which ensures stem cell renewal and tissue homeostasis. Linking these processes acts like a fail-safe mechanism.82 AJUBA acts as a negative regulator of Wnt signalling pathway (Figure 2C). GSK-3β is responsible for phosphorylating β-catenin, tagging it for degradation. AJUBA binds to both β-catenin and GSK-3β, thus promoting β-catenin phosphorylation through proximity.83 Thus, AJUBA leads to the degradation of β-catenin. Further studies show that upon stimulation of Wnt signalling pathway AJUBA undergoes proteasomal degradation.83 Suggesting that AJUBA acts as a molecular switch between Wnt-β-catenin and Hippo-YAP induced proliferation. However, the exact relationship between AJUBA-Wnt-Hippo remains elusive.

4.2 | Epidermal differentiation to maintain stratified epidermis

4.2.1 | Notch signalling

Notch signalling is responsible for differentiation and maintaining the differentiated squamous layers. NOTCH receptors are
transmembrane proteins that are proteolytically cleaved upon binding to JAG1/2 or DLL1/3/4 ligands. The cleaved NOTCH intra-cellular domain (NICD) enters the nucleus, binds the DNA-binding protein RBPJ, and regulates gene expression. Recently, AJUBA was shown to directly bind NOTCH1/2, NICD1/2, and NUMB, a negative regulator of Notch signalling, in primary mouse keratinocytes (Figure 3A). AJUBA promotes differentiation by selectively sequestering NUMB, thereby blocking NUMB's association with NOTCH/NICD and NUMB-mediated recruitment of the E3 ubiquitin-protein ligase ITCH to NOTCH/NICD, which is required for NOTCH/NICD's proteasomal degradation (Figure 3A). Upon ligand-induced NOTCH activation, AJUBA knockout keratinocytes showed impaired nuclear translocation of NICD1/2 and transcription of canonical NOTCH downstream targets. Together, these data suggest that AJUBA permits cells to undergo differentiation by sequestering NUMB and permitting NOTCH transcriptional targets to be upregulated.

The depiction of NOTCH, Hippo and WNT signalling not being correlated (section 3.1.3) may also connect to AJUBA regulation and it would be interesting to explore cross-talk between AJUBA, NOTCH, Hippo and WNT, especially in cellular decision-making during epidermal stratification.

### 4.2.2 Cell adhesion mediated epidermal remodelling

Adherens junctions are cadherin-catenin complexes that link the cytoskeleton to the plasma membrane and form cell-cell linkages throughout the stratified epidermis. E-cadherin, the most abundant cadherin within the epidermis, forms a complex between β-catenin and p120-catenin to α-catenin, which holds F-actin in place. Desmosomes are other cell junction complexes that link desmosomal cadherins (desmocollin and desmoglein) with the catenin paralogs, plakoglobin and plakophilin, to intermediate filaments of keratin to strengthen the epidermis and have been implicated in oral lesions, thickened skin, and severe blistering when expression is altered. Alterations in adhesion and cytoskeletal components within the epidermis leads to specific stratification phenotypes. Specifically, loss of E-cadherin within the epidermis leads to hyperproliferation of the basal layer, loss of p120-catenin leads to altered epidermal inflammatory responses, loss of β-catenin leads to hair follicle defects (while plakoglobin replaces β-catenin within the epidermis to maintain stratification) and loss of α-catenin is linked to loss of epidermal cell polarity and leads to the dissociation of cells. Little overlap in phenotypes suggests important independent roles for maintaining keratinocyte differentiation.

In primary mouse keratinocyte, AJUBA was shown to interact with α-catenin and is recruited to cadherin-dependent cell-cell adhesion complexes (Figure 3B). AJUBA also interacts directly with F-actin through its PreLIM domain, while simultaneously binding to α-catenin (Figure 3B). Keratinocytes from AJUBA-null mice exhibit abnormal cell-cell junction formation and/or stability and function.

In addition, AJUBA has been shown to dynamically interact with several cellular adhesion molecules during remodelling and migration to control Rac activation. In actin remodelling, AJUBA induces Rac activation and maintains E-cadherin adhesion in human keratinocytes. AJUBA is required to stabilize pre-formed adherens junctions by recruiting F-actin (Figure 3C). F-actin-AJUBA complex binds to activated Rac via the PreLIM domain on AJUBA (Figure 3C). Stability of actin-remodelling promotes cellular migration via p130Cas activation of Rac1. AJUBA binds p130Cas through the LIM domain, bringing it in proximity of Rac1, permitting activation and promoting migration. Loss of AJUBA correlated with a loss of p130Cas complexes and AJUBA-null keratinocytes demonstrated a PAK1-dependent reduction in migration linked to a decrease in Rac localization at cellular adhesion complexes. Rac1 can also be regulated by Cdc42, a GTPase that when recruited destabilizes junctions; an opposing effect of p130Cas.

These data identify AJUBA as a new component at cadherin-mediated cell-cell junctions and suggest that AJUBA may contribute to the bridging of the cadherin adherence junctions to the actin cytoskeleton and as such contribute to the formation or strengthening of cadherin-mediated cell-cell adhesion. Overall, AJUBA functions as a molecular switch impinging on cellular fate through a multitude of mechanisms in a context-dependent nature.

### 5 AJUBA'S ROLE IN CANCER STEM CELLS OF EPIDERMAL CANCERS

Cancer stem cells are a small subpopulation of cells within tumors with self-renewal as well as differentiation capabilities and can initiate a tumor when transplanted into a recipient animal. This section will explore AJUBA as a double-edged sword in epidermal cancer stem cell biology, with tumor-suppressive and tumor-promoting functions (Figure 4 and Table 1).

#### 5.1 AJUBA functions as tumor suppressor in head and neck cancer

Head and neck squamous cell carcinoma (HNSCC) affects ~600,000 patients per year worldwide, and approximately half of patients die from advanced disease within 5 years. HNSCC arises in the moist mucosal lining of the upper aerodigestive tract. Human papilloma virus (HPV) infection is a risk factor associated with HNSCC. The most common genetic mutations in HNSCC patients are p53 (71%), FAT1 (23%), CDKN2A (22%), PIK3CA (18%), NOTCH1 (17%), and HRAS (6%). AJUBA loss-of-function mutations are seen in 7.5% and 18% of HNSCC and cutaneous squamous cell carcinoma...
cSCC cases, respectively. Further examination revealed that an additional 11.1% of human HNSCC cases have an allelic loss at chromosome 14, encompassing AJUBA. Allelic loss correlated with reduced expression of AJUBA-protein within human tumors and conventional mouse experiments showed that AJUBA functions as haploinsufficient tumor suppressor especially in the oral mucosa but also in the epidermis. Significantly, AJUBA mutations and allelic loss are found in a mutual exclusive pattern with mutations of NOTCH1/2/3 receptors or ADAM10, a metalloproteinase essential for ligand-induced cleavage of NOTCH receptors (Figure 4). Together, these data indicate that alterations of AJUBA alterations might contribute to ~18% of HNSCC and cSCC by blocking proper Notch signalling and that NOTCH inactivation is a hallmark of HNSCC and cSCC initiation.

**Figure 4** AJUBA functions as a tumor suppressor or promoter in cancers throughout the human body. Brief illustrations summarizing AJUBA’s involvement in different organs during tumor initiation, growth and metastasis. This schematic highlights the context-dependent manner in which AJUBA influences molecular signalling pathways throughout the human body. Whereby, green boxes represent tumor-promoting roles and red boxes represent tumor-suppressive roles of AJUBA. AJUBA functions within signalling pathways to promote cancer. In epidermal squamous cell carcinoma (ESCC), AJUBA stabilizes Grb2 promoting RAS/ERK-dependent MMP10/13 expression, increasing migration and invasion. In colon cancer, AJUBA promotes migration by upregulating N-cadherin via Smad1/SNAIL. AJUBA promotes cancer within the nucleus through its role as a co-activator. In breast cancer, AJUBA acts as a co-activator of estrogen receptor (ER) to promote cellular growth. In pancreatic cancer, AJUBA generates a positive feedback loop between SP1/GC transcription of EGFR to increase cellular proliferation. AJUBA was shown to be influenced by miRNA in lung cancer leading to increased invasive and migratory cellular properties. AJUBA has also been linked to patient drug resistance in cervical cancer. As a tumor suppressor, loss of AJUBA in head and neck squamous cell carcinoma (HNSCC) affects the Notch signalling pathway, loss of AJUBA increases growth and invasive properties of cells in hepatocyte cancer by linking E-cadherin and Hippo signalling, and lastly in prostate cancer, androgen receptor causes miRNA-dependent depletion of AJUBA leading to increased migration and metastasis.
| Cancer type | Mechanism | Cancer hallmark | Model used | Citation |
|-------------|------------|----------------|------------|----------|
| Breast cancer | AJUBA is a co-activator of oestrogen receptor alpha, increasing TFF1, Greb1 and SGK3 | ↑ proliferation | T47D and MCF7 cell lines | Xu et al\textsuperscript{109} |
| Cervical cancer | Patients showed high AJUBA levels in tumors and correlated to increased nuclear-YAP | ↑ drug resistance | Various stages of cervical patient tumor biopsies and tissue samples SiHa and HeLa cervical cancer cell lines | Bi et al\textsuperscript{111} |
| Colon cancer | Smad1 promotes AJUBA/Snail controlling E-Cadherin | ↑ cell migration | Human colon cancer cell lines: HCT116, SW1116 and 293 Ts Patient tissue samples: colorectal adenocarcinoma specimens | Yang et al\textsuperscript{107} |
| | AJUBA expression increased MAPK cascade and Wnt signalling pathway | ↑ Proliferation, differentiation & EMT | SW480, SW620 and HCT116 colon cancer cell lines | Dommann et al\textsuperscript{106} |
| | AJUBA decreases IFIT2 by binding JAK1 preventing STAT1 phosphorylation | ↑ cell survival | HEK293 T, SW−1116, Caco−2 and colorectal adenocarcinoma patient-derived cell lines. Xenografted SW−1116 cells into C57Bl/6 mice | Jia et al\textsuperscript{113} |
| | Decrease of miR−1184, increased downstream target AJUBA to inactivate Hippo signalling | ↑ tumor initiation | SW480, HCT116 and human colorectal cancer tumor cells Xenograft mice – human colorectal cancer tumor cells | Wang et al\textsuperscript{104} |
| ESCC | AJUBA-overexpression increased nuclear YAP | ↑ cell growth & chemoresistance | SGC−7901 and NCI-N87 cells | Li et al\textsuperscript{105} |
| | Overexpression of AJUBA increased MMP10/13, which upregulated MAPK/ERK1/2 pathway | ↑ migration & ↑ cell growth | ESCC cell lines: KYSE30, KYSE70, KYSE140, KYSE180, KYSE410, KYSE450, KYSE510 and HEK293 T cells | Shi et al\textsuperscript{103} |
| Lung cancer | miR−193b−3p and −5p are decreased, and shown to directly increase of AJUBA expression | ↑ migration, proliferation & colony formation | Brain metastatic lung cancer (PC14PE6/LvBr4) and AS49 cell lines | Choi et al\textsuperscript{110} |
| PDAC | Patients with high Ajuba and SP1 expression had poorer prognosis. Ajuba is a co-activator of SP1, and Ajuba itself is a target gene of SP1 | ↓ survival, ↑ metastatic potential | Human PDAC tissues, HEK−293 T and PanC1 cells | Zhang et al\textsuperscript{108} |

Ajuba as a tumor suppressor

| ESCC | Increased AJUBA expression correlated to a decrease of activated YAP | ↓ cell growth | Human patient samples | Du et al\textsuperscript{101} |
| HNSCC | AJUBA loss correlated to an expansion of undifferentiation basal keratinocytes through inactivated NOTCH-signalling | ↓ differentiation | HNSCC patient-derived cell lines and primary murine keratinocytes, CRISPR-mediated Ajuba-knockout mice | Loganathan et al\textsuperscript{33} |

(Continues)
AJUBA's functions in oesophageal squamous cell carcinoma

Oesophageal cancer is the 8th most frequently diagnosed cancer worldwide and oesophageal squamous cell carcinoma (ESCC) is the most common subtype, accounting for over 90% of cases.97 Smoking and alcohol consumption is associated with increased risk.99 The only familial link is a genetic abnormality at chromosome 17q25.99,100 The most common genetic mutations in ESCC patients are p53 (95%), NOTCH1(16%), KMT2D (11%), PIK3CA (11%), FAT1 (10%), NFE2L (10%).101 In an exome sequencing study, AJUBA was mutated in 7% of cases and 8 different mutations were identified within the LIM domain leading to lower expression levels of AJUBA.102 Consistently, a genomic analysis in 2017 depicted a mutational frequency of AJUBA LIM domain at 3.9% of ESCC, either a stop mutation or a frameshift mutation in either LIM1/2.101 Within this study, they also found that expression level of AJUBA was lower in AJUBA-mutated tumor samples than in the wild-type tumor samples.101 Interestingly, an increase in AJUBA expression was inversely correlated with YAP1-phosphorylation suggesting AJUBA functions as a tumor suppressor in ESCC (Figure 4).102

However, another study reports that AJUBA was significantly overexpressed in ESCC tumor samples in comparison to adjacent non-tumor tissue.103 shRNA knockdown of AJUBA prevented ESCC cell growth and xenograft growth in BALB/c-nu mice. Furthermore, they demonstrated that AJUBA depletion decreases migration of ESCC cells and overexpression consistently led to an increase in migration. To define a mechanism, this study completed RNA sequencing experiments. MMP10 and MMP13 were downregulated in AJUBA-knockdown ESCC cells. MMP10/13 are metalloproteases that induce expression of pro-inflammatory proteins and collagenase 3, respectively. Further exploration revealed that overexpression of AJUBA in ESCC cells upregulated MMP10/13 via MAPK/ERK1/2 pathway, similarly to its role in oocyte development. Together, this suggests that in this context AJUBA is promoting tumor growth.

AJUBA's function in other cancers

In addition to squamous cell carcinomas of the skin and the upper aerodigestive tract, AJUBA has been implicated in several epithelial cancers, working through a multitude of mechanisms summarized in Table 1.

AJUBA can function as an oncogene to promote cell proliferation and migration. In colorectal cancer AJUBA was found to be a downstream target of miR-1184. AJUBA binds and sequesters LATS, thereby inactivating Hippo signalling and promoting colorectal cancer, in response to decreased expression of miR-1184.104 AJUBA overexpression inhibited Hippo signalling, thereby upregulating nuclear YAP. This AJUBA-induced YAP increase permits regulation of cyclin D1, Bcl-xL and GLUT1, influencing mitochondrial potential and glucose uptake to increase cell growth and chemoresistance.105 Additionally, AJUBA induces metastatic colon cancer by promoting migratory pathways in human colon cancer cells such as Smad1 induced AJUBA/Snail binding to downregulate E-cadherin expression.106,107 Furthermore, AJUBA functions as a co-activator to promote proliferation in pancreatic and breast cancer with the transcription factor SP1 via a feed-forward loop of downstream targets, EGFR and IGF1R and with ERα by recruiting DBC1 and CBP/p300 to induce expression of TFF1, Greb1 and SGK3 (ERα target genes), respectively. Similar promotion of migration is seen in lung cancer. Precisely, miR-193b-3p and −5p inhibition led to increased metastatic potential and both miRNA’s decreased AJUBA expression resulting in a decrease in migration, proliferation and colony-forming

| Cancer type            | Mechanism                                                      | Cancer hallmark       | Model used                                                                 | Citation            |
|------------------------|----------------------------------------------------------------|-----------------------|-----------------------------------------------------------------------------|---------------------|
| Hepatocyte cancer      | Overexpression of AJUBA diminished both β-catenin and YAP levels| ↓ cell growth         | 293 T, COS7, Hep3B, HepG2, Huh7, SK-Hep1, SMMC7721, SNU449, MHCC97H, MHCC97L, HCCLM3 and BEL7402 cell lines | Liu et al112        |
| SCLC                   | Decrease in YAP-dependent hippo signalling decreases AJUBA, correlating to shorter survival in patients | ↑ Cytoskeletal rearrangement, ↑ morphological changes | SCLC patient tissue samples BEAS−2B, A549, and NCI-H441 lung adenocarcinoma cells, HCl-H209, Lu134A, Lu134B, Lu139, SBC3 and SBC5 SCLC cells | Horie et al. (2016)114 |
| Prostate cancer        | miR−193a−3p binds androgen receptor and reduces expression of AJUBA | ↑ migration and metastasis | LNCaP and C4-2B cells | Jia et al113        |

Notes: Summary of the mechanistic details and correlating model organisms utilized in the discovery of AJUBA's involvement as a tumor promoter and inhibitor.

Abbreviations: ESCC, oesophageal squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; SCLC, small cell lung cancer.
activity of lung cancer cell lines. Lastly, cervical cancer patients displaying high AJUBA expression positively correlated with resistance to cisplatin treatment via YAP/TAZ upregulation.

AJUBA can also functions as a tumor suppressor in some cancers inhibiting cell growth and migration by modulating several cellular components and proteins. AJUBA overexpression was shown to inhibit hepatocellular carcinoma cell proliferation by diminishing β-catenin and YAP levels, preventing their translocation and expression of Cyclin D1 and CYR61, respectively. Loss of AJUBA also enhanced migration and is correlated to prostate cancer metastasis. In prostate cancer, overexpression of miR-193a-3p, an androgen receptor binding miRNA, induces migration of LNCaP and C4-2B cells by downregulating its target AJUBA.

These studies highlight the complexity in pinpointing the exact mechanism of AJUBA in a cancer context (Figure 4) and lead to more questions – does AJUBA function through multiple signalling pathways and mechanisms and is its function dependent on the genetic make-up of a given tumor?

6 | CONCLUSION

The adaptor protein AJUBA functions as a key molecular switch fine-tuning several key signalling pathways within the epidermis from epidermal morphogenesis to maintenance of the adult epithelium. Due to the highly intricate pathways, a challenge remains in connecting the overarching pathway choices that AJUBA makes to decide an epidermal cells’ fate. AJUBA’s effects during tumorigenesis are tissue-specific and context-dependent, whereby both the under- and overexpression of AJUBA can lead to tumorigenesis and metastatic promotion. This review functionally connects the diverse roles of AJUBA in the precise development of the epidermis, maintaining adult epidermal integrity, and its numerous roles in development of various cancers.

ACKNOWLEDGMENTS

This work was supported by a project grant from the Ontario Research Fund Research Excellence Round 8 (RE08-065).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

KS wrote the manuscript under the supervision and guidance of DS. All authors who contributed to the article have read and approved the final manuscript.

ORCID

Daniel Schramek © https://orcid.org/0000-0001-9977-2104

REFERENCES

1. Watt FM, Estrach S, Ambler CA. Epidermal Notch signaling: differentiation, cancer and adhesion. Curr Opin Cell Biol. 2008;20:171-179.
2. Fuchs E. Skin stem cells: rising to the surface [Internet]. J Cell Biol. 2008;180:273-284.
3. Fujiwara H, Tsutsui K, Morita R. Multi-tasking epidermal stem cells: Beyond epidermal maintenance [Internet]. Dev Growth Differ. 2018;60:531-541.
4. Blanpain C, Fuchs E. Epidermal homeostasis: A balancing act of stem cells in the skin. Nat Rev Mol Cell Biol. 2009;10:207-217.
5. Gudjonsson JE, Johnston A, Dyson M, et al. Mouse models of psoriasis [Internet]. J Invest Dermatol. 2007;127:1292-1308.
6. Wong VW, Sorkin M, Glotzbach JP, Longaker MT, Gurtner GC, Surgical Approaches to Create Murine Models of Human Wound Healing Journal of Biomedicine and Biotechnology 2011;2011:969618. http://dx.doi.org/10.1155/2011/969618
7. Wei J, Edwards GA, Martin DJ, Huang H, Crichton Michael L., Kendall MAV, Allometric scaling of skin thickness, elasticity, viscosity and stiffness for micro-medical device translation: from mice, rats, rabbits, pigs to humans Scientific Reports 2017;7(1):15885. http://dx.doi.org/10.1038/s41598-017-15830-7
8. Moriya M, Durham AD, Moriya M, et al. Multiple roles of notch signaling in the regulation of epidermal development. Dev Cell. 2008;14:594-604.
9. Joost S, Annsusver K, Jacob T, et al. The molecular anatomy of mouse skin during hair growth and rest. Cell Stem Cell. 2020;26:441-457.
10. Estrach S, Cordes R, Hozumi K, et al. Role of the Notch ligand Delta1 in embryonic and adult mouse epidermis. J Invest Dermatol. 2008;128:825-832.
11. Blanpain C, Lowry WE, Pasolli HA, et al. Canonical notch signaling functions as a commitment switch in the epidermal lineage. Genes Dev. 2006;20:3022-3035.
12. Nowell C, Radtke F. Cutaneous notch signaling in health and disease. Cold Spring Harb Perspect Med. 2013;3:a017772.
13. Negri VA, Logtenberg M, Rein LM, et al. Delta-like-1-mediated cis-inhibition of Jagged1/2 signalling inhibits differentiation of human epidermal cells in culture. Sci Rep. 2019;9:1-11.
14. Totaro A, Castellan M, Battilana G, et al. YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate. Nat Commun. 2017;8:1-13.
15. Estrach S, Legg J, Watt FM. Syntenin mediates Delta1-induced cohesiveness of epidermal stem cells in culture. J Cell Sci. 2007;120:2944-2952.
16. Proksch E, Brandner JM, Jensen J-M. The skin: an indispensable barrier [Internet]. Exp Dermatol. 2008;17:1063-1072. https://doi.org/10.1111/j.1600-0625.2008.00786.x
17. Sarpal R, Yan V, Kazakova L, et al. Role of α-Catenin and its mechano-sensing properties in regulating Hippo/YAP-dependent tissue growth [Internet]. PLoS Genet. 2015;11:e1004854.
18. Rognoni E, Wallo G. The roles of YAP/TAZ and the hippo pathway in healthy and diseased skin. Cells. 2019;8:411.
19. Zhang H, Pasolli HA, Fuchs E. Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. Proc Natl Acad Sci USA. 2011;108:2270-2275.
20. Pocaterra A, Romani P, Dupont S. YAP/TAZ functions and their regulation at a glance [Internet]. J Cell Sci 2020;133:jcs230425.
21. Codella VA, Sun G, Irvine KD. Regulation of YAP by mechanical strain through Jnk and Hippo signaling. Curr Biol. 2014;24:2012-2017.
22. Lamar JM, Xiao Y, Norton H, et al. SRC tyrosine kinase activates the YAP/TAZ axis and thereby drives tumor growth and metastasis [Internet]. J Biol Chem 2019;294:2302-2317.
23. Rausch V, Hansen CG. The hippo pathway, YAP/TAZ, and the plasma membrane [Internet]. Trends Cell Biol. 2020;30:32-48. https://doi.org/10.1016/j.tcb.2019.10.005
24. Thompson BJ. YAP/TAZ: Drivers of tumor growth, metastasis, and resistance to therapy. BioEssays. 2020;42:1900162.
69. Sun G, Irvine KD. AJUBA family proteins link JNK to hippo signaling [Internet]. Sci Signal. 2013;6:a81.
70. Prigent C, Giet R. Aurora A and mitotic commitment. Cell. 2003;114:531-532.
71. Hirota T, Kunitoku N, Sasayama T, et al. Aurora-A and an interacting activator, the LIM protein AJUBA, are required for mitotic commitment in human cells. Cell. 2003;114:585-598.
72. Torchia EC, Zhang L, Huebner AJ, et al. Aurora kinase-a deficiency during skin development impairs cell division and stratification. J Invest Dermatol. 2013;137:78-86.
73. Bai M, Ni J, Wu J, et al. A novel mechanism for activation of Aurora-A kinase by AJUBA. Gene. 2014;543:133-139.
74. Bai M, Ni J, Shen S, et al. Aurora-A kinase-inactive mutants disrupt the interaction with AJUBA and cause defects in mitotic spindle formation and G2/M phase arrest in HeLa cells. BMB Rep. 2014;47:631-636.
75. Ferrand A, Chevrier V, Chauvin J-P, et al. AJUBA: a new microtubule-associated protein that interacts with BUBR1 and Aurora B at kinetochores in metaphase. Biol Cell. 2009;101:221-240.
76. Satyanarayana A, Kaldis P. Mammalian cell-cycle regulation: Several cdks, numerous cyclins and diverse compensatory mechanisms. Oncogene. 2009;28:2925-2939.
77. Diril MK, Ratnacaram CK, Padmakumar VC, et al. Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. Proc Natl Acad Sci USA. 2012;109:3826-3831.
78. Santamaría D, Barrière C, Cerqueira A, et al. Cdk1 is sufficient to drive the mammalian cell cycle. Nature. 2007;448:811-815.
79. Chen X, Stauffer S, Chen Y, et al. AJUBA phosphorylation by CDK1 promotes cell proliferation and tumorigenesis. J Biol Chem. 2016;291:14761-14772.
80. Nicolas M, Wolfer A, Raj K, et al. Notch1 functions as a tumor suppressor in mouse skin. Nat Genet. 2003;33:416-421.
81. Azzolin L, Zanconato F, Bresolin S, et al. Role of TAZ as mediator of wnt signaling. Cell. 2012;151:1443-1456.
82. Azzolin L, Panciera T, Soligo S, et al. YAP/TAZ incorporation in the β-catenin destruction complex orchestrates the Wnt response. Cell. 2014;158:157-170.
83. Haraguchi K, Ohsugi M, Abe Y, et al. AJUBA negatively regulates the Wnt signaling pathway by promoting GSK-3β-mediated phosphorylation of β-catenin. Oncogene. 2008;27:274-284.
84. McGill MA, McGlade CJ. Mammalian Numb proteins promote Notch1 receptor ubiquitination and degradation of the Notch1 intracellular domain. J Biol Chem. 2003;278:23196-23203.
85. Sumigray KD, Lechner T. Cell adhesion in epithelial development and barrier formation. In: Current Topics in Developmental Biology, Massachusetts, MA: Academic Press, 2015;112:383-414. https://doi.org/10.1016/bs.ctdb.2014.11.027
86. Livshits G, Kobilak A, Fuchs E. Governing epithelial homeostasis by coupling cell - Cell adhesion to integrin and growth factor signaling, proliferation, and apoptosis. Proc Natl Acad Sci USA. 2012;109:4886-4891.
87. Miroshnikova YA, Le HQ, Schneider D, et al. Adhesion forces and cortical tension couple cell proliferation and differentiation to drive epidermal stratification. Nat Cell Biol. 2018;20:69-80.
88. Brousard JA, Koetsier JL, Green KJ. Desmosomes pattern cell mechanics to govern epidermal tissue form and function [preprint]. bioRxiv 2020;2020.01.21.914176. https://doi.org/10.1101/2020.01.21.914176
89. Yin T, Green KJ. Regulation of desmosome assembly and adhesion [Internet]. Semin Cell Dev Biol. 2004;15:665-677.
90. Michels C, Buchta T, Bloch W, et al. Classical cadherins regulate desmosome formation [Internet]. J Invest Dermatol. 2009;129:2072-2075.
91. Brandner JM, Zorn-Kruppa M, Yoshida T, et al. Epidermal tight junctions in health and disease. Tissue Barriers. 2015;3:e974451.
92. Nola S, Daigaku R, Smolarczyk K, et al. AJUBA is required for Rac activation and maintenance of E-cadherin adhesion. J Cell Biol. 2011;195:855-871.
93. McCormack JJ, Brutsche S, Ouadda ABD, et al. The scaffold protein AJUBA suppresses CdkGAP activity in epithelia to maintain stable cell-cell contacts. Sci Rep. 2017;7:9249. https://doi.org/10.1038/s41598-017-09024-4
94. Shen L, Shi Q, Wang W. Double agents: genes with both oncogenic and tumor-suppressor functions. Oncogenesis. 2018;7:1-14.
95. Pai SI, Westra WH. Molecular pathology of head and neck cancer: implications for diagnosis, prognosis, and treatment [Internet]. Annu Rev Pathol. 2009;4:49.
96. Lawrence MS, Sougnez C, Lichtenstein L, et al. Comprehensive genomic characterization of head and neck squamous cell carcinomas [Internet]. Nature 2015;517:576-582.
97. Wheeler JB, Reed CE. Epidemiology of esophageal cancer. Surg Clin North Am. 2012;92:1077-1087.
98. Tran GD, Di SX, Abnet CC, et al. Prospective study of risk factors for esophageal and gastric cancers in the Linxian General Population Trial cohort in China. Int J Cancer. 2005;113:456-463.
99. Enzinger PC, Mayer RJ. Esophageal Cancer [Internet]. N Engl J Med. 2003:349:2241-2252.
100. Risk JM, Mills HS, Garde J, et al. The tyrosin esophageal cancer (TOC) locus: More than just a familial cancer gene. Dis Esophagus. 1999;12:173-176.
101. Du P, Huang P, Huang X, et al. Comprehensive genomic analysis of Oesophageal Squamous Cell Carcinoma reveals clinical relevance. Sci Rep. 2017;7:1-9.
102. Gao YB, Chen ZL, Li JG, et al. Genetic landscape of esophageal squamous cell carcinoma. Nat Genet. 2014;46:1097-1102.
103. Shi X, Chen Z, Hu X, et al. AJUBA promotes the migration and invasion of esophageal squamous cell carcinoma cells through upregulation of MMP10 and MMP13 expression. Oncotarget. 2016;7:36407-36418.
104. Wang X, Chen Y, Liu W, et al. Hsa_circ_0128846 promotes tumorigenesis of colorectal cancer by sponging hsa-miR-1184 and releasing AJUBA and inactivating Hippo/YAP signalling [Internet]. J Cell Mol Med. 2020;24:9908-9924.
105. Li H, Fu L, Liu B, et al. AJUBA overexpression regulates mitochondrial potential and glucose uptake through YAP/β-catenin/GLUT1 in human gastric cancer [Internet]. Gene 2019;693:16-24.
106. Dommann N, Sánchez-Taltavull D, Eggs L, et al. The LIM protein ajuba augments tumor metastasis in colon cancer [Internet]. Cancers (Basel). 2020;12:1913.
107. Yang D, Hou T, Li L, et al. Smad1 promotes colorectal cancer cell migration through AJUBA transactivation [Internet]. Oncotarget. 2017;8:110415-110425.
108. Zhang B, Song L, Cai J, et al. The LIM protein AJUBA/SP1 complex forms a feed forward loop to induce SP1 target genes and promote pancreatic cancer cell proliferation [Internet]. J Exp Clin Cancer Res. 2019;38:205. https://doi.org/10.1186/s13046-019-1203-2
109. Xu B, Li Q, Chen N, et al. The LIM protein AJUBA recruits DBC1 and CBP/p300 to acetylate ERα and enhances ERα target gene expression in breast cancer cells [Internet]. Nucleic Acids Res. 2013:1:13-14.
110. Choi KH, Shin CH, Lee WJ, et al. Dual-strand tumor suppressor miR-193b-3p and -5p inhibit malignant phenotypes of esophageal cancer cell migration [Internet]. Biosci Rep. 2019;39:BSR20190634. https://doi.org/10.1042/BSR20190634
111. Bi L, Ma F, Tian R, et al. AJUBA increases the cisplatin resistance of esophageal squamous cell carcinoma cells [Internet]. J Invest Dermatol. 2020;15:665-677.
113. Jia L, Gui B, Zheng D, et al. Androgen receptor-regulated miR-NA-193a-3p targets AJUBA to promote prostate cancer cell migration [Internet]. Prostate 2017;77:1000-1011.

114. Horie Masafumi, Saito Akira, Ohshima Mitsuhiro, Suzuki Hiroshi I., Nagase Takahide, YAP and TAZ modulate cell phenotype in a subset of small cell lung cancer Cancer Science 2016;107(12):1755-1766. http://dx.doi.org/10.1111/cas.13078