REVIEW

RNA-Binding Proteins in Female Reproductive Pathologies

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RNA-binding proteins are key regulatory molecules involved primarily in post-transcriptional gene regulation of RNAs. Post-transcriptional gene regulation is critical for adequate cellular growth and survival. Recent reports have shown key interactions between these RNA-binding proteins and other regulatory elements, such as miRNAs and long noncoding RNAs, either enhancing or diminishing their response to RNA stabilization. Many RNA-binding proteins have been reported to play a functional role in mediation of cytokines involved in inflammation and immune dysfunction, and some have been classified as global post-transcriptional regulators of inflammation. The ubiquitous expression of RNA-binding proteins in a wide variety of cell types and their unique mechanisms of degradative action provide evidence that they are involved in reproductive tract pathologies. Aberrant inflammation and immune dysfunction are major contributors to the pathogenesis and disease pathophysiology of many reproductive pathologies, including ovarian and endometrial cancers in the female reproductive tract. Herein, we discuss various RNA-binding proteins and their unique contributions to female reproductive pathologies with a focus on those mediated by aberrant inflammation and immune dysfunction. (*Am J Pathol* 2017, 187: 1200–1210; <http://dx.doi.org/10.1016/j.ajpath.2017.01.017>)

Post-transcriptional gene regulation (PTGR) is an imperative process for the overall health of eukaryotic cells and mRNAs. With recent advancements in sequencing and proteomics screening techniques, >1500 RNA-binding proteins (RNABPs) have been identified to interact with all classes of RNAs.¹ These RNABPs form ribonucleoprotein complexes with RNAs and proteins, which are crucial units involved in gene regulation.² Most RNABPs are ubiquitously expressed in higher than average levels compared to other proteins.¹ Post-transcriptional regulation of mRNA has a downstream function in various signaling pathways.³ Many RNABPs have diverse binding specificity and have been reported to interact with RNAs encoding for functionally relevant genes.⁴ Similar to miRNAs, many of these RNABPs coordinate regulation of related mRNAs.^{5–7} Mutations, which result in aberrant expression or functioning of these RNABPs, highlight the importance of these regulatory molecules in many processes central to adequate cellular health, including their contribution to pathologies.

Next-generation sequencing and other advanced profiling techniques and biochemical methods, such as photo-activatable ribonucleoside-enhanced cross-linking and immunoprecipitation, have revealed the abundance and diversity of RNABPs and their interactions with multiple RNA subclasses.⁸ The classification of RNABPs by their interacting RNA targets has allowed for the isolation of specific PTGR pathways involved in human diseases. Although much of the published research on RNABPs has focused on mRNA binding proteins, there are other RNABPs that play a role in PTGR through their interaction with noncoding RNAs (ncRNAs).¹ These ncRNAs may become encoded into ribonucleoprotein complexes, and therefore serve as guides to direct the ribonucleoprotein complexes to search out target transcripts and genome loci.⁹

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Gerstberger et al¹ elegantly generated a census of human RNABPs analyzed for their interactions with different classes of RNA and characterized 122 RNABPs that interact with ncRNAs, including miRNAs, P-element induced wimpy testis (PIWI)-interacting RNAs, and long noncoding RNAs. These ncRNAs, along with their corresponding RNABPs, can control multiple aspects of post-transcriptional mRNA processing via degradation, silencing, or activation of gene loci. For example, miRNAs repress mRNA stability through their association with Argonaute proteins,¹⁰ whereas PIWI-interacting RNAs protect the genome from genomic instability by interacting with PIWI proteins.⁹ Thus, the relationship between these ncRNAs, RNABPs, and their respective PTGR pathways provides a greater dimension of complexity when analyzing the role of RNABPs in disease pathologies.

In the female reproductive tract, inflammation is part of the ovulation process and normal endometrial tissue remodeling during menstruation. Inflammation is also necessary for embryo implantation and downstream events in a successful pregnancy.¹¹ Simply put, these processes are tightly regulated by coordinated actions of cytokines, chemokines, decoy receptors, and immune cell networks. These inflammatory mediators are influenced by female sex hormones, estrogen and progesterone.¹² Inflammation has also been implicated in most reproductive pathologies.^{12,13} Uncontrolled, delayed, or dysregulated delicate homeostatic inflammatory processes have all been demonstrated to contribute to disease pathogenesis in the reproductive tract, including endometriosis and pregnancy loss.^{11,14}

Herein, we present an overview of RNABPs that interact with RNAs or other post-transcriptional regulators in the context of inflammation-mediated female reproductive pathologies. We focus on the function of these RNABPs as well as their mechanisms in cancer and non-cancer reproductive pathologies. For the purpose of this review, we will restrict contributions of RNABPs to the pathophysiology of endometriosis, endometrial and ovarian cancer, and pregnancy loss.

Overview of Post-Transcriptional Gene Regulation Mechanisms

The formation of a type of ribonucleoprotein complex, the RNA-induced silencing complex, is required for miRNA-mediated gene silencing.¹⁵ The miRNA-induced silencing complex is composed of miRNAs, which are directly bound by members of the Argonaute protein family.¹⁶ The association of miRNA-induced silencing complex with an mRNA via base pairing allows for the mediation of both mRNA decay and translational repression through the recruitment of various enzyme complexes.^{15,17} Deadenylases are first recruited, followed by cytoplasmic mRNA degradation in the 3'-5' or 5'-3' direction by the RNA exosome or exoribonuclease 1, respectively.^{18,19} Many proteins involved in the 5'-3' mRNA degradation pathway

concentrate in granular cytoplasmic foci known as processing bodies, which have also been shown to colocalize with various miRNA-mediated RNA decay factors.^{17,18} The export of the mRNAs from the nucleus to the cytoplasm is a necessary process for the aforementioned degradation pathway mechanisms to successfully occur. Some RNABPs, such as the Tristetraprolin (TTP; ZFP36) family, exhibit autoregulatory negative feedback loops via binding to their own regulatory elements, such as 3' untranslated regions, and cause degradation of their own mRNA.^{20,21} P-body assembly is dependent on the pool of mRNA to be decayed and is induced in response to stresses or other conditions that lead to the inhibition of translation initiation.²² In addition, stress granules are cytoplasmic structures that also form during certain types of cellular stress.¹⁷ Translationally repressed mRNAs aggregate within these stress granules, which are then sorted and delivered to processing bodies for degradation.¹⁸ Both processing bodies and stress granules contain various shared components, including mRNA, eukaryotic translation initiation factor 4E, micro-ribonucleoproteins, and regulators of mRNA stability and translation such as RNABPs.²³ Thus, processing bodies and stress granules are dynamic structures that have been reported to play a critical role via the interplay between RNABPs, mRNA decay, and translation.

Modulation of Immune-Inflammation Axis by RNABPs

RNABPs and their role in reproductive pathologies is an emerging field. It has been shown that RNABPs regulate inflammation and immune cell functions, and as a result are implicated in inflammation-associated pathologies. In this review, we have summarized the role of RNABP in inflammation and immune cell modulation because a delicate balance between inflammatory mediators and sex hormones is key to successful female reproductive events.²⁴ The role of RNABPs in inflammation and immune cell biology has been highlighted in the context of selected reproductive pathologies.

Modulation of Inflammation by HuR/TTP Axis RNABPs

Given that most of the previous investigations on inflammation modulation by RNABPs have been focused on the human antigen-R (HuR)/TTP axis, this review will primarily highlight this axis.^{25,26} Recently, TTP has been classified as a global post-transcriptional regulator of feedback control in inflammation.²⁷ Both HuR and TTP are expressed in the nucleus and cytoplasm of a wide variety of cell types of the reproductive tract, including innate immune cells, such as macrophages. TTP activation is dependent on p38-activated MAP kinase-activated protein kinase-2 phosphorylation at the site of serine 52 and 178 in the mouse²⁸ and 60 and 186 in humans by extracellular signal regulated kinase 2 protein kinase.²⁹

Phosphorylation of TTP allows preferential binding of 14-3-3 protein in the cytoplasm. The formation of this complex renders TTP ineffective as a destabilizing protein as it leads to the reduction of TTP affinity to AU-rich elements (AREs) on inflammatory cytokine transcripts.^{28,30,31} Conversely, some *in vitro* studies have demonstrated that phosphorylation has no impact on ARE-binding—mediated degradation by TTP.³² Phosphorylation status of TTP also has been reported to be associated with CCR4-Not1 deadenylation complex and HuR/TTP MAP kinase-activated protein kinase-2 phosphorylation modulation of ARE-dependent translation.³¹ Non 14-3-3-bound TTP can bind to the 3' untranslated region AREs of inflammatory cytokines, such as tumor necrosis factor

α (TNF- α) and mediate degradation via 5'-3' and 3'-5' pathways aforementioned above (Figure 1).

Taylor et al³³ documented that TTP-deficient mice generated by targeted disruption of *Zfp36* gene, although normal at birth, developed systemic autoinflammatory phenotype within 1 to 8 weeks after birth. The hyper-inflammatory phenotype was primarily attributed to elevated circulating levels of TNF- α as injection of neutralizing TNF- α antibodies reversed the inflammatory phenotype in TTP-deficient mice. Additional studies in TTP-deficient mice led to the identification of granulocyte-macrophage colony-stimulating factor as another physiological target of TTP.²⁵ Furthermore, TTP expression is correlated with severity of

Figure 1 Modulation of inflammation by the HuR/TTP axis. The balance between HuR and TTP is shifted in response to external stress stimuli. Under normal conditions (left), P38 activates protein phosphatase 2a (PP2a), which catalyzes the dephosphorylation and activation of TTP. At the same time, PP2a deactivates serine/threonine protein kinase Chk2 (Chk2), which allows cyclin-dependent kinase 1 (Cdk1) to phosphorylate HuR and render it inactive. Phosphorylated HuR can be translocated to the cytoplasm, tipping the balance in favor of TTP, which can bind the mRNAs of inflammatory cytokines and promote their degradation. Under stress conditions (right), P38 activates mitogen-activated protein kinase-activated protein kinase 2 (MK2), which phosphorylates and deactivates TTP and allows it to be translocated to the cytoplasm. At the same time, P38 activates Chk2, which can phosphorylate and deactivate Cdk1. The inhibition of Cdk1 allows HuR to remain dephosphorylated, which adds to the transport of TTP out of the nucleus, tips the balance in favor of HuR, which can bind the mRNAs of inflammatory cytokines and stabilize them to promote their translation, thus promoting the inflammatory response. There are numerous additional players in this pathway that help maintain this balance. **Dashed arrow** indicates inhibited binding. AAAAA, poly A tail; ARE, AU rich element; ORF, open reading frame; P, phosphorylation; P-bodies, processing bodies.

rheumatoid arthritis, ulcerative colitis, and systemic lupus erythematosus in humans.²⁶ On the other side of the axis, HuR can bind to most of the same cytokines possessing AREs, such as TNF- α , and stabilize the transcripts. Much like TTP, HuR nucleocytoplasmic shuttling and activation are in part dependent on p38 mitogen-activated protein kinase-mediated phosphorylation,³⁴ and phosphorylation by checkpoint kinase 2,³⁵ protein kinase C,³⁶ and cyclin-dependent kinase 1 can influence binding activity of HuR on inflammatory mRNAs.³⁷ Because NF- κ B controls many cytokines involved in inflammation, it is not surprising that the TTP/HuR axis is involved in regulation of several NF- κ B signaling-dependent proinflammatory cytokines. Tiedje et al²⁷ have elegantly demonstrated that a phosphorylation-dependent inactivation of TTP is required for NF- κ B signal transduction. In addition, TTP can impair NF- κ B/p65 nuclear translocation in an ARE-mediated decay-independent manner and can ultimately result in TTP-induced cell death.^{27,38} These data clearly suggest that the HuR/TTP axis is a critical regulator of physiological inflammation, and any perturbations in this axis will potentially lead to autoimmune and inflammatory conditions.

A high number of RNABPs expressed in the reproductive tract regulate the expression of genes encoding inflammatory molecules, either promoting or controlling inflammation.³⁹ For example, roquin-1,⁴⁰ regnase-1,⁴¹ and tristetraprolin²⁷ are RNABPs that attenuate inflammatory responses via target mRNA degradation, whereas HuR is often suggested to oppose TTP, thereby promoting inflammation through target mRNA stabilization.⁴² Mice with germline knockout of TTP display a severe inflammatory phenotype with myeloid hyperplasia and autoimmunity due to excessive TNF production, indicating the role of TTP in the regulation of TNF transcript expression.⁴³ Conversely, the role of HuR in PTGR has been less straightforward. Although HuR participates in the transcript stabilization and expression of proinflammatory cytokine IL-17 in a mouse model of experimental autoimmune encephalomyelitis, it can also act as a negative regulator of macrophage-mediated inflammation.⁴⁴ Overexpression of HuR was shown to act as a translational silencer for TNF, IL-1 β , and transforming growth factor- β 1, whereby the production of these proteins was decreased after lipopolysaccharide (LPS) challenge in mice compared to controls that did not overexpress HuR.⁴⁵ Some studies have linked interactions between HuR and miRNAs via shared mRNA functional binding sites.⁴⁶ Indeed, competitive miRNAs, such as miR-637, have been reported to also vie for binding of inflammatory molecules.⁴⁷

Modulation of Immune Cell Development by RNABPs

In nonpathological conditions, immune cell populations are dynamic and differentiate normally.⁴⁸ RNABPs participate in the development and differentiation of lymphoid cells, such as T and B cells. In particular, conditional double knockout of ZFP36L1 and ZFP36L2 in lymphocytes lead to the development of T-lymphoblastic leukemia in double-

knockout mice, whereas single knockout of either gene displays no developmental defect, indicating a unique multi-RNABP pathogenesis-dependent role in lymphocytes.⁴⁹ The development of T-lymphoblastic leukemia is attributed to the increased production of Notch homolog 1 (Notch1), elucidating a novel transcript target for ZFP36L1 and ZFP36L2 and the importance of both RNABPs in the developmental regulation of T lymphocytes in the thymus.⁴⁹ The RNABP HuR is also involved in the developmental programming of both T and B cells. Conditional knockout of HuR in thymocytes elucidates its role in thymopoiesis as a cell cycle suppressor and modulator of proapoptotic signals.⁵⁰ In B cells, the production of HuR protein is up-regulated three-fold on stimulation with LPS or with anti-CD40 and IL-4 and IL-5 *in vitro* compared to unstimulated B cells.⁵¹ Furthermore, B-cell-specific conditional knockout of HuR leads to significantly lower production of all types of immunoglobulin isotypes in the serum of mice, except for IgA when compared to control mouse, indicating that HuR is necessary for antibody production after antigenic challenge.⁵¹

The RNABPs TIA1 cytotoxic granule associated RNA binding protein (TIA-1) cytotoxic granule-associated RNABP and TTP prevent pathological overexpression of TNF- α via inhibition of translation by TIA-1 and degradation of TNF- α transcripts by TTP. Deletion of TIA-1 in mice results in mild arthritis, whereas deletion of TTP results in severe arthritis, as well as cachexia, dermatitis, and autoantibody formation.³³ Mice containing deletions in both TIA-1 and TTP develop severe arthritis.⁵² RNABP experiments investigating immunomodulation by RNABPs indicate that TIA-1/TTP knockout murine macrophages secrete lower TNF- α protein when stimulated by LPS, indicative that these RNABPs are not only involved in PTGR, but can alter cell variation, which can further exasperate inflammatory pathologies.⁵²

The Importance and Contributions of Gene Regulation by RNABPs in Reproduction and Associated Pathologies

Although the roles of many RNABPs are commonly focused on inflammatory pathways, RNABPs also appear to have a significant role in the initiation and regulation of normal reproductive events. In the female reproductive tract specifically, a number of RNABPs are involved in reproductive events, including but not limited to oocyte maturation, ovulation, endometrial tissue remodeling, embryo implantation, placenta development, and successful pregnancy. We have provided a summary of RNABP contributions in female reproductive pathologies in [Figure 2](#).

RNABPs in Embryonic Growth and Placental Development

RNABPs, including ZFP36/TTP as well as its less-studied family members, ZFP36L1 (Tis11b), ZFP36L2 (Tis11d), and

Figure 2 Contributions of RNA-binding proteins (RNABPs) in female reproductive pathologies. The dysregulation of the immune inflammation axis in endometriosis, fetal loss, infertility, and ovarian and endometrial cancer, is mediated in part by RNABPs, which have been shown to be differentially regulated in animal models as well as humans. AUF1, AU-rich element-binding factor 1; eIF4G1, eukaryotic translation initiation factor 4 γ 1; TIA-1, TIA1 cytotoxic granule associated RNA binding protein.

the rodent-specific ZFP36L3, are readily detectable in placental tissues and yolk sac in humans and in mice.^{53,54} Indeed, we found differential expression of TTP family members in a first-trimester human trophoblast cell line, HTR-8/SVNeo between normoxic and hypoxic conditions, suggesting an abundance of TTP family members in placenta and their potential regulation under hypoxia (K.K., C.T., unpublished data). In fact, RAW264.7 macrophages stimulated with potent inflammatory trigger lipopolysaccharide, under prolonged hypoxic conditions, led to reduced TNF- α protein levels. This decrease in TNF- α was mediated by TTP-dependent destabilization of TNF- α mRNA via a 3'-untranslated region-dependent mechanism, suggesting a regulatory role for TTP in hypoxia-dependent resolution of inflammation.⁵⁵ Using a knockout mouse model of rodent-specific ZFP36L3, a number of growth factors, including heparin-binding epidermal growth factor, endothelial lipase Lipg, and type 1 transferrin receptor, which is responsible for

iron uptake by the placenta for the developing fetus, were shown to be likely targets of ZFP36L3.⁵⁴ In addition, ZFP6L2 has been shown to be critical for female fertility because of the crucial role of RNABPs in early embryonic development. The amino-terminal truncated form of ZFP36L2 causes arrest in early embryonic development at the two-cell stage. Disruption of the ZFP36L1 gene in mice results in an embryonic lethal phenotype at the embryonic day 11 because of disruptions in chorioallantoic fusion.⁵⁶ Recent evidence suggests that HuR/embryonic lethal, abnormal vision-like 1 (ELAVL1) elicits transcriptional and post-transcriptional changes in mRNAs important for extraembryonic (placental and yolk sac) as well as embryonic development.⁵⁷ Using ELAVL1 knockout mice (embryonic lethal), the initiation of branching and processes of labyrinth morphogenesis, which are imperative for placental development, are absent from the knockout model, suggesting HuR/ELAVL1 is involved in this initiation of branching.⁵⁷

RNABPs in Ovulation, Oocyte Maturation, and Female Fertility

RNABP mRNAs are expressed in the reproductive tract of humans and mice and are influenced by menstrual/estrous cycles.^{58,59} Female mice with mutations in ZFP36L2 were unable to ovulate even under pharmacological conditions, and attempts to mature oocytes from these mice *ex vivo* was hindered because of meiotic arrest imposed by cAMP.⁶⁰ Using the TTP knockout mouse model, it has also been demonstrated that TTP is critically involved in mouse meiosis, chromosomal arrangement, spindle morphology, and polar body extrusion, all of which are critical events for oocyte maturation.⁶¹ Meiotic asymmetry and a lack of oocyte polarization significantly decreases reproductive success in females and likely contributes to infertility.⁶² Furthermore, ablation of the Src associated in mitosis of 68 kDa (Sam68) RNABP induces sterility and infertility in females because of its essential role in oocyte maturation and its involvement in the expression of gonadotropin receptors in the ovary.⁶³ These data clearly provide evidence that disruptions in RNABPs impede female fertility.

Post-Transcriptional mRNA Regulation by RNABPs in Endometriosis and Pregnancy Loss

Endometriosis, a proinflammatory gynecological disease, is characterized by the growth of the endometrial-like tissue outside of uterine cavity. Research to understand the immunological basis behind disease establishment has elucidated aberrant inflammation and immune system dysfunction in endometriosis patients.⁶⁴ The innate arm of the immune system, such as macrophages, is polarized toward the M2 phenotype, which specializes in tissue repair, instead of tissue clearance via phagocytosis.⁶⁵ In addition, increased concentration of inflammatory cytokines in the biological fluids from patients further strengthens the notion of the involvement of immune system in the development of endometriosis.⁶⁶ Inflammatory cytokines have also been directly implicated to initiate symptoms of pain and infertility in endometriosis patients.^{67,68} Aberrant activation of RNABPs may participate in this process via PTGR of transcripts encoding for proinflammatory cytokines.

Recent work from our group has indicated dysregulation of the HuR/TTP axis in patient ectopic lesions compared to matched eutopic endometrium obtained from women with endometriosis (K.K., S.H.A., M. Bidarimath, Y. Nasirzadeh, S.S. Singh, A.T. Fazleabas, S.L. Young, B.A. Lessey, M.K., C.T., unpublished data). In addition, we have demonstrated differential responses of inflammatory cytokines in endometriotic and epithelial endometrial cell lines, suggesting a potential functional role of the HuR/TTP axis in the pathophysiology of endometriosis. Women with endometriosis present decreased TIA-1 and HuR expression, both of which are RNABPs responsible for modulation of proinflammatory cytokines, in the eutopic and ectopic endometrium compared to normal healthy controls.^{59,69} Decreases in regulatory

molecules of inflammation likely contribute to the pathogenesis and pathophysiology of endometriosis by contributing to the inflammatory milieu in the peritoneal cavity and symptoms of pain and infertility observed in endometriosis patients. Another RNABP that has been implicated in the pathogenesis of endometriosis is Musashi-1.⁷⁰ This stem cell marker is frequently associated with tumorigenesis and abnormal growth; however, it has also been found to be elevated in the eutopic endometrium of endometriosis patients.⁷⁰ Finally, the RNABP heterogeneous nuclear ribonucleoprotein D0, AUF-1, which mediates the destabilization of the mRNA of DNA methyltransferase 1, is lower in the eutopic endometrium of endometriosis patients.⁷¹ Together, correlative evidence of these RNABPs shows promise and points toward involvement via inflammation and immune-modulatory pathogenesis mechanisms. However, many additional studies are required to understand the molecular mechanism of RNABP contributing to the pathogenesis of endometriosis.

Recurrent pregnancy loss (RPL) is a multifactorial disorder for patients who experience pregnancy failure in the first trimester more than two or three times.⁷² Aberrant maternal inflammation has been linked with thrombosis during fetal loss, and fetal distress molecular pathways influence fetal health outcome. Our group has demonstrated that the TTP family may be involved in spontaneous fetal loss in a porcine model of pregnancy loss.⁷³ Currently, there are no reports on the direct involvement of RNABPs with recurrent pregnancy loss in humans, but given their presence at the maternal-fetal interface and regulation of several key inflammatory processes, it is logical to assume that RNABPs potentially participate in the pathogenesis of RPL. We have recently shown that TTP and HuR RNABPs are expressed in the placenta, and TTP is involved in the modulation of inflammation-induced RPL via aberrant expression in an LPS-induced mouse model of RPL.⁷⁴ In addition, our group has demonstrated differential localization patterns of TTP in placentas obtained from LPS-treated mice.⁷⁴ Collectively, we believe that these RNABPs are participating in pathogenesis of pregnancy loss, including RPL via aberrant nucleocytoplasmic localization shuttling and expression.

Overall, it is apparent that a dysregulation of RNABP contributes to increases in reproductive tract inflammation and immune dysfunction and ultimately contributes to pathogenesis of reproductive pathologies. However, the role of RNABP across different cell types of the female reproductive pathologies is not well characterized and requires further investigation to understand the molecular mechanisms underlying the relationship between RNABP and specific reproductive pathologies.

Gene Regulatory Mechanisms and RNABPs Implicated in Endometrial and Ovarian Cancer

RNABPs play important roles in RNA regulation in tumor carcinogenesis and development. Recent research has gone

into unraveling RNABP involvement in cancers, including endometrial and ovarian cancer. Although many studies have been correlative and have focused on clinical outcomes and normal tissue compared to carcinoma expression levels, increasingly literature is starting to unveil unique mechanistic interactions between RNABPs and other regulatory factors during cancer development and transformation. Epithelial ovarian cancer is the most deadly gynecological disease,⁷⁵ with a median survival of 2 to 4 years.⁷⁶ Endometrial carcinoma is the most common gynecological malignancy worldwide, and in recent decades the incidence of endometrial carcinoma has been increasing.⁷⁷

Sam68 is a KH domain RNABP involved in a wide range of cellular processes, including signal transduction, transcription, RNA metabolism, cell cycle progression, and apoptosis.^{78,79} Sam68 features six proline-dominated sequences and a tyrosine-rich area at its C terminus.⁷⁸ Signaling proteins that include SH2 or SH3 domains bind at these important regions.⁷⁸ The activity of Sam68 may be altered via tyrosine hydroxylase, which interrupts homodimerization, leading to reduced affinity for RNA *in vitro*.⁸⁰ This RNABP has been associated with poor prognostic roles in a wide variety of cancers, including non-small-cell lung cancer,⁸¹ bladder cancer,⁸² esophageal squamous cell carcinoma,⁸³ colorectal cancer,⁸⁴ and aggressive neuroblastoma.⁸⁵ Expression of Sam68 at both the mRNA and protein levels in both epithelial ovarian cancer⁷⁹ and endometrial carcinoma is significantly higher in pathological tissue when compared to para-tumor tissue and normal tissue.⁸⁶ The elevated expression of Sam68 in endometrial cancer is also significantly associated with histological grade, International Federation of Gynecology and Obstetrics stage, and myometrial invasion.⁸⁶ Sam68 has a proliferative effect on tissues, as demonstrated via siRNA knockdown experiments of this gene in both human endometrial adenocarcinoma (Ishikawa)⁸⁶ and epithelial ovarian cancer (OVCAR-3) cells.⁷⁹ Furthermore, random homozygous knockout-induced Sam68 deficiencies in nude mice lead to tumor-suppressing effects and increased metastatic tumors.⁸⁷ It has also been suggested that its role is dependent on a range of factors, including context and type of tissue.⁸⁸ Determining the biological mechanisms of Sam68 could be valuable as we potentially progress to viewing Sam68 in a therapeutic sense.

In addition to noncancer reproductive pathologies, HuR has been extensively studied with regard to its roles in a variety of cancers, including epithelial ovarian cancers.⁸⁹ In the cytoplasm, HuR stabilizes mRNA, aiding to translation of its bound transcripts, and may act as a tumor maintenance gene.⁹⁰ HuR has been shown to be involved in proliferation, apoptosis, angiogenesis, metastasis, invasion, and chemotherapy resistance,⁸⁹ all of which are tightly linked to cancer pathology. In addition to being involved in inflammation regulation covered earlier, HuR regulates many mRNAs that are hallmarks of cancer progression, such as p53, vascular endothelial growth factor, cyclins, and cyclooxygenase 2.⁹¹ Furthermore, HuR expression is significantly increased in

both poorly differentiated and advanced stage endometrial carcinomas.⁹¹ Indeed, knockdown of HuR results in decreased cell proliferation and induction of apoptosis in *in vitro* models using Ishikawa cells.⁹⁰ In an investigation of HuR associated with ovarian cancer, HuR was found to be present in the nucleus in most cases.⁹² This nuclear HuR was significantly linked with invasive and high-grade cancers as well as poor disease-free survival.⁹² Nuclear HuR intensity, through multivariate analysis, was demonstrated to be an independent prognostic factor for poor disease-free survival.⁹² Together, these data point toward altered phosphorylation status of HuR, resulting in potentially higher inactive HuR that likely contributes to aberrant inflammation in endometrial carcinomas.

Evidence of complex miRNA-RNABP networks has been reported in ovarian cancer pathogenesis. HuR has also been implicated in ovarian cancer by stabilizing long noncoding RNAs, which contribute to its pathogenesis.⁹³ NEAT1, a long noncoding RNA, is up-regulated in ovarian cancer, both within cell lines and patient samples, and HuR has been reported to preserve NEAT1's structural integrity.⁹³ Specifically in metastatic high-grade serous ovarian carcinomas, HuR mRNA and protein levels are widely expressed and mRNA levels are connected to poor survival outcomes in high-grade serous ovarian carcinoma patients.⁹¹

La-related protein 1 (LARP1) is an evolutionarily conserved RNABP, and has been associated with many cancers.⁹⁴ LARP1 knock-down studies have revealed that LARP1 is necessary for both cancer cell survival and chemotherapy resistance.⁹⁴ *In vivo*, this RNABP incites tumor formation.⁹⁴ It increases the amount of available mRNA of genes involved in cell survival, including B-cell lymphoma 2, human epidermal growth factor receptor 3, and Rac-γ serine/threonine-protein kinase, and reduces the amount of available proapoptotic genes, such as *BIK*, *TNFA*, and *DAPK2*.⁹⁴ It is pertinent to note that within ovarian cancer patients, the tumor's response to chemotherapy is a vital determinant of survival, which makes LARP1 particularly relevant in this kind of cancer.⁹⁴ This notion is strengthened by the fact that patients with the highest LARP1 mRNA expression have reported significantly worse outcomes, and a heightened risk of death at any time.⁹⁴ RNABP eukaryotic translation initiation factor 4 γ 1 is a subunit of the eukaryotic translation initiation complex, and it aids in ensuring the establishment of the mRNA-ribosome by recruiting mRNA to the ribosome.⁹⁵ This is a critical process, as the formation of this complex is the rate-limiting step of the initiation stage of mRNA translation.⁹⁵ The expression of eukaryotic translation initiation factor 4 γ 1 in serous ovarian cancer at both the mRNA and protein level is significantly up-regulated and associated with clinical tumor stage.⁹⁵ In addition, patients with low eukaryotic translation initiation factor 4 γ 1 protein reported longer survival time.⁹⁵ Together, findings of these key RNABPs outline the clinicopathological relevance of these RNABPs in reproductive cancers.

In addition to dysregulated RNABP expression, aberrant miRNA expression has also been demonstrated in ovarian cancer pathogenesis.^{96,97} Direct connections with miRNAs and RNABPs have been reported with respect to the Drosha and DGCR8 core components in miRNA biogenesis microprocessor, because RNABPs can confer specificity for recruiting and processing different pri-miRNAs.⁹⁸ It has been shown that RNA binding molecules p68,⁹⁹ p72,⁹⁹ KSRP,¹⁰⁰ and TAR DNA-binding protein 43¹⁰¹ all can specifically modulate miRNA maturation via interactions with the miRNA processing complexes. RNABP human DEAD-box protein has been demonstrated to be a regulatory modulator of miRNAs involved in DNA damage via its modulatory effects on the Drosha processing of a select group of miRNAs.¹⁰² Inhibition of this RNABP results in ovarian tumor growth and metastasis in mice and poor clinical outcomes in human patients with serous ovarian cancer.¹⁰² In addition, RNABPs Lin28B and insulin-like growth factor 2 have been shown to be correlated in ovarian cancer.¹⁰³ Although much progress has been made to determine this association between miRNAs and RNABPs in cancer, further studies will need to further elucidate links between their cancer-specific interactions and cancer progression. Moving forward, research in this field may be crucial in finding molecular targets for selected therapies, determining prognosis, and addressing chemoresistance.

RNABPs as Potential Therapeutic Targets

Because of their ubiquity, RNABPs at the clinical level have not been used for biomarker detection. Currently, preclinical trials using compounds that attenuate or up-regulate RNABPs have yielded promising findings. Nanotherapy-based drug delivery approaches have become a feasible type of treatment of cancers in the past few years and most recently have been shown to be useful for dermatologic-related pathologies.¹⁰⁴ Moreover, nanoparticles have been used in carrying DNA fragments and RNA inhibitors, such as siRNA, and these options may change the future of treating pathologies.¹⁰⁴ The improvement in our understanding of HuR has led to novel therapeutic exploration. Cy-3–labeled folic acid–derivatized DNA dendrimer nanocarriers are used as a targeted delivery system of siRNAs. These nanocarriers contain siRNAs for HuR and have already been demonstrated to be successful in suppressing tumor growth in mice with ovarian cancer and significantly increasing their lifespan.¹⁰⁵ In addition, HuR nanotherapy in combination with a CXCR4 antagonist has been reported to control lung cancer metastasis via suppression of CXCR4–stromal cell–derived factor 1 axis.¹⁰⁶ These encouraging results support the continuation of research in this specific field of targeted cancer therapies. However, similar to all mRNA regulators, including miRNAs, specificity to both the gene of interest and site of the targeted biological pose

challenges. Indeed, the need to understand the roles of RNABPs in different cell populations has been previously highlighted.^{105,107} Furthermore, long-term benefits of RNABP biologics have not been evaluated and could also prove to be limiting, especially because of the fact that these are protein-based therapeutics and will likely not be able to be delivered as oral biologics. RNABPs such as the HuR/TTP axis, which primarily target inflammatory cytokines, are early to intermediate responders; thus, targeting this axis may not translate to long-term biologics. Another important aspect to take into consideration is that potential non-specificity is minimized as nanoparticles can be specifically delivered to the target tissue or organ. In a pharmacological sense, early to intermediate elements usually result in homeostatic changes in the patient short-term, but over time, the homeostatic changes can revert because of other mechanisms, such as miRNA families with the same inflammatory targets being up-regulated in response to down-regulation induced by biologics. When taken together, it comes as no surprise then that RNABPs have been identified as therapeutic targets to treat inflammatory diseases; however, additional research is required to validate the feasibility of this new type of treatment using nanotechnology.

Since the first anti-TNF biological therapy was authorized by the US Federal Drug Administration, current TNF inhibitors have shown efficacy in dampening inflammation. These drugs are mainly either monoclonal antibodies or fusion proteins that confer the characteristic of being injectable and disintegrate when digested. Indeed, many other therapeutic targets have been developed since, including anti-IL-23, IL-1, IL-6, and interferons, among others, to treat a wide variety of inflammatory pathologies, such as rheumatoid arthritis, psoriasis, multiple sclerosis, and pediatric autoimmune disorders.¹⁰⁸ However, modulation of inflammatory response is one of the many key points to address severe immunosuppression as well as serious adverse events. Because of these reasons, these therapeutics have a limited safety profile and patients are monitored carefully.¹⁰⁷ Despite the success of biological therapies worldwide, there are still some unmet needs regarding the treatment of immune- and inflammation-mediated disorders. Nevertheless, TTP overexpression in the mouse model has demonstrated the capability to protect mice from autoimmune disorders, which suggest the benefit of a drug that simulates TTP may be a good agent to treat inflammation-mediated pathologies, including reproductive tract pathologies.¹⁰⁹

Conclusions

In this review, we highlighted the importance of RNABPs and their contributions to reproductive pathologies. We also sought to highlight unique mechanisms, with an emphasis on the HuR/TTP axis, that these RNABPs influence their

target genes to exert their effects in a wide variety of cell types as well as potential use as therapeutic targets. Their unique involvement with NF- κ B in regulation of inflammation, cell senescence, and proliferation further highlights their importance in maintaining a homeostatic balance. Furthermore, unique cancer-specific functions of some RNABPs may be at play in endometrial and ovarian cancer. We believe that, although the reproductive field lacks knowledge on RNABPs compared to other molecules involved in PTGR such as miRNAs, RNABPs contribute to these pathologies as much as miRNA networks. Because of the intricate roles of RNABPs in maintaining homeostasis, we believe that rather than evaluating RNABP families in separation, they should be investigated as a ratio. Future investigations should evaluate the unique interplays between PTGR mechanisms shared or in tandem with RNABPs and other regulators as well as the unique roles of RNABPs on translation, success, or failure of the targets, and their unique contributions to reproductive tract pathologies.

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