Long Non-Coding RNAs in Choriocarcinoma

Choriocarcinoma (CC), a subtype of trophoblastic disease, is a rare and highly aggressive neoplasm. There are two main CC subtypes: gestational and non-gestational, (so called when it develops as a component of a germ cell tumor or is related to a somatic mutation of a poorly differentiated carcinoma), each with very diverse biological activity. Long non-coding (lnc) RNAs are non-coding transcripts that are longer than 200 nucleotides. LncRNAs can act as oncogenes or tumor suppressor genes. Deregulation of their expression has a key role in tumor development, angiogenesis, differentiation, migration, apoptosis, and proliferation.

1. Introduction

Choriocarcinoma (CC), a subtype of trophoblastic disease [1], is a rare and highly aggressive tumor with varied global incidence [2]. CC is histologically characterized by an admixture of: syncytiotrophoblasts and mononucleated cytotrophoblastic cells. Two significant CC subtypes, gestational and non-gestational (so called when it develops as a component of a germ cell tumor or is related to a somatic mutation of a poorly differentiated carcinoma) (Figure 1), have diverse underlying biologies and clinical outcomes (Table 1) [3]. The former can arise in the uterus and rarely in extrauterine sites following a hydatidiform mole (50%), a miscarriage or induced abortion (25%), a normal pregnancy (22.5%), or an ectopic pregnancy (2.5%) [2]. Non-gestational CCs have been shown to arise from pluripotent germ cells in both male and female gonads, most commonly in the ovary and retroperitoneum [3][4][5], or pelvis [6]. Pure CC is exceedingly rare, but focal areas of CC are seen in approximately 12% of embryonal and teratocarcinomas [7]. Foci of CC may be present in gestational mixed trophoblastic tumors and in non-gestational mixed germ cell tumors. Rare cases of extrauterine carcinoma with trophoblastic cells represent differentiation of pluripotent cells into malignant somatic cells [3].
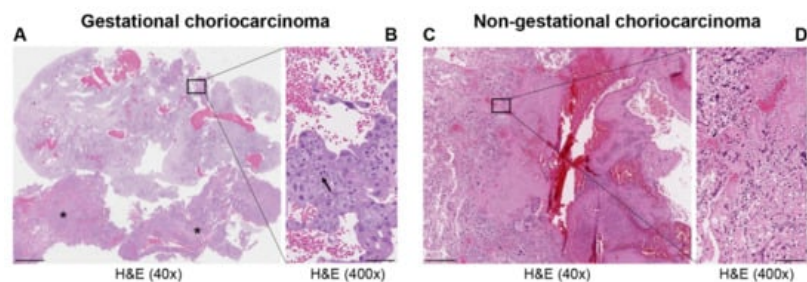

Figure 1. Representative images of hematoxylin and eosin (H&E) staining in gestational (**A**,**B**) and non-gestational (**C**,**D**) choriocarcinoma. Uterine curettage (**A**,**B**); (**A**) low power (40×; scale bar = 500 μm) with abundant decidualized endometrium and blood and two large clusters of choriocarcinoma (*); (**B**) high power (400×; scale bar = 50 μm) shows aggregates of large trophoblasts with marked atypia and prominent mitotic figures (arrow) covered by atypical syncytiotrophoblasts. Invasive choriocarcinoma in the ovary (**C**,**D**). (**C**) Component of a germ cell tumor of the ovary with large and atypical trophloblastic cells with hemorrhage and necrosis (low power 40×; scale bar = 500 μm) associated with dysgerminoma (not present in the picture). (**D**) Markedly atypical syncíotrophoblasts and cytotrophoblasts (high power 400×; scale bar = 50 μm).

Table 1. Summary of the features of choriocarcinoma—adapted from Cheung et al. [3].

Types	Gestational Choriocarcinoma	Non-Gestational Choriocarcinoma	
		Germ Cell Tumor	Somatic Carcinoma
Incidence	Ranges from 1 in Europe to −9.2 in Asia/40,000 pregnancies	Rare < 1% of all ovarian tumors —children, young adults but rarely in older adults. Midline tumors mostly in males	Rare ovarian carcinomas in adults
Origin	It may develop as a complication of pregnancy, usually following a complete mole	It arises from primordial germ cells	It arises from differentiation of pluripotent cells into a somatic carcinoma
Site	Primarily uterus and also intraplacental; rarely ovary and extrauterine sites	Gonads, midline: pineal gland, mediastinum, retroperitonum	Lung, gastrointestinal tract, and other organs, including very rare ovarian carcinoma and uterine cases in post-menopause
Histopathology	Mononuclear cytotrophoblast and intermediate trophoblast and multinucleated syncytiotrophoblast cells with marked atypia and mitoses	Mainly in pure form with cyto- and syncytiotrophoblast or with other components of germ cell tumors (mixed germ cell tumor)	Presence hCC-producing multinucleated giant cells; transition with co-existing somatic carcinoma of the particular organ
Cytogenetic features	Deletion of 7p12-7q11.2; amplification of 7q21-q31 and loss of 8p12-21 [3]	Gain of 12p [3]	Unknown
Biochemical features	hCG in serum or urine (>10 × 10^3 mIU/mL)	hCG in serum or urine	hCG in serum or urine— variable
Molecular markers	Upregulation of *TP53*, *CDKN1A*, *RB1*, *EGFR*, *ERBB2*, *c-MYC*, *BCL2*, *NANOG*, *H19* [3][8]; Downregulation of *NECC1*, *TIMP3*, *DOC-2/hDab2*, *RASSF1A*, *CDKN2A*, *CDH1*, *IGF2*, *OCT4*, *SOX2* [3][8]; Mutated genes: *NLRP7*, *ARID1A*, *SMARCD1*, *EP300* [9]	Upregulation of *CGB5*, *CGA*, *NANOG*, *STELLA*, *GDF3* [3]	Upregulation of *NANOG* [3]
Treatment	Chemotherapy	Surgery is indicated. Chemotherapy of different drug regimens is applied	Surgery is indicated. May respond to chemotherapy but it may not be useful
Prognosis	Good	Poor	Poor

2. Dysregulated lncRNAs in Choriocarcinoma

LncRNAs, by acting as tumor suppressor genes or oncogenes, play a critical physiological role in apoptosis, invasion, metastasis, and cell proliferation in several cancers. LncRNAs are also involved in the pathogenesis of cancers of the female reproductive system (ovarian, uterine, vaginal, cervical, and vulvar cancers) [10]. However, to date, only a few studies address the role of lncRNA in CC. Novel mechanistic insights into how gene expression is specifically regulated by lncRNAs, contributing to CC formation, are outlined below. **Table 2** summarizes the functions of the main lncRNAs implicated in CC.

Table 2. LncRNAs with a role in choriocarcinoma.

LncRNA	Locus	Role	Molecular Functions	Target Pathway	Sources
MALAT1	11q13.1	Oncogene	Sponge of miR-218	Unknown	Three CC cell lines, JEG-3, JAR, and BeWo cells, and a normal cell line human trophoblast cells (HT cells) [11]
H19	11p15.5	Oncogene	Unknown	PI3K/AKT/mTOR [12]	Placenta, androgenetic moles, and choriocarcinoma [13]; CC cell line JEG-3, including MTX- and 5-FU-resistant variants [12]
MEG3	14q32.3	Tumor suppressor	Unknown	Unknown	Placenta; 4 cell-lines associated with pregnancy, including HTR-8/SVneo, JEG-3, WISH, and HUVEC [14]

LncRNA	Locus	Role	Molecular Functions	Target Pathway	Sources
PCA3	9q21-22	Oncogene	Sponge of miR-106b	Unknown	Three CC cell lines, JAR, BeWo, and JEG-3, and the human chorionic trophoblast cell HTR-8 [15]
LINC00261	20p11.21	Tumor suppressor	Unknown	Unknown	Sixty CC tissues and 60 adjacent non-cancerous tissues; 3 CC cell lines, namely, BeWo CCL-98, JEG-3, and JAR [16]
OGFRP1	22q13.2	Oncogene	Unknown	AKT/mTOR	Two CC cell lines, JEG-3 and JAR [17]
MIR503HG and LINC00629	Xq26	Tumor suppressor	Unknown	Unknown	RNA samples from a commercial normal human tissue panel and 18 cancer cell lines, JEG-3 cell line [18]

3. Clinical Applications of lncRNAs in Choriocarcinoma

Given that lncRNAs can be detected in almost all tissues and body fluids, including peripheral blood, and are not easily degraded by RNases, they can be more sensitive and specific than DNA, proteins, and protein-coding RNAs in the diagnosis of tumors [19][20]. Thus, lncRNAs can be potentially utilized as novel non-invasive biomarkers in cancer diagnosis [21]. Even though there has been increasing interest in the study of lncRNAs in gynecological cancers, research is still preliminary. The association of many dysregulated lncRNAs with clinical features leads to the design of novel biomarkers for diagnosis and management of patients with gynecological cancers, ultimately attaining better prognosis [10]. Whether lncRNAs will be also beneficial in the early detection of CC needs to be investigated. Currently, there is no evidence that lncRNAs are used as biomarkers for diagnosis and prognosis of patients with CC. Moreover, given the rarity of the tumor, we hope that these molecules may be used in the future to distinguish gestational CC from non-gestational tumors in an easier and quicker way than the current state-of-the-art methods (biopsy and microsatellite analysis).

4. LncRNAs as Therapeutic Targets

Interestingly, most lncRNAs are expressed in a cell type- and tissue-specific manner, and in this respect, lncRNAs lend themselves as potentially important therapeutic targets. Specifically, lncRNA targeted therapy, unlike chemotherapeutic regimens, needs to hinder the signaling pathways in which lncRNA plays an important role in tumor development and progression, whilst aiming to avoid the adverse effects on normal cells. There may also be a potential role of lncRNA as biomarkers for disease management, including non-invasive screening [22].

Recent advances utilize CRISPR technology and oligonucleotide-based therapy to modify gene expression. These approaches may be pivotal for developing novel therapeutic approaches aiming at interfering with oncogenic lncRNAs [23].

The use of RNA interference (RNAi) to modulate gene expression has been successfully used to silence lncRNA in vivo [24]. Advancements in RNAi have led to Food and Drug Administration (FDA) approval of lipid nanoparticles (LNPs) which contain short interfering RNA (siRNA) that is being used to treat hereditary amyloidosis in adults [25].

LncRNAs associated with cancer can also be modulated by using antisense oligonucleotides (ASOs). ASOs are single stranded with a central native or chemically modified DNA stretch, flanked on either side by RNA nucleotides. DNA forms RNA/DNA heteroduplex with target lncRNA, which is cleaved by endogenous RNaseH1 [26]. ASOs have been successfully used to alter mRNA expression as part of the treatment of various diseases, including different types of cancer where lncRNAs are highly expressed [27][28].

Advances in genomic interference methods, with superior specificity when compared to RNAi, have been developed recently. CRISPR interference (CRISPRi) using CRISPR-Cas9 and CRISPR-Cas13 led to the successful silencing of transcriptionally active lncRNA-expressing sites. In the CRISPR-Cas9 approach, nucleotides devoid of nucleolytic activity, termed dead-Cas9, are fused to transcriptional repressors. Transcriptional silencing is achieved because, via guide RNAs, this fusion protein is targeted to a specific gene promoter [29]. The development of guide RNAs targeting the promoters of thousands of lncRNAs in the human genome together with CRISPRi enabled the selective inactivation of lncRNA genes in human cancer cell lines. Liu et al. further underscored tissue specificity of lncRNA by identifying approximately 500 lncRNAs in only one cell type [30]. Therefore, it is possible to achieve transcriptional silencing of lncRNAs via CRISPR-based approaches, however, several challenges limit their immediate use for therapeutic targeting [31][32][33][34]. Major

limitations include delivery methods (viral and non-viral vectors), with viral vectors limited in the size of the cargo they are able to deliver to the cells of interest [35][36][37][38]. Regardless of the current limitations, the future undoubtedly looks bright for CRISPR-directed lncRNA therapies.

5. Future Directions and Conclusions

LncRNAs acting as tumor biomarkers have been shown to have an important role in the diagnosis and management of a number of cancers. Studies on the relationship between lncRNAs and gynecological cancer are still at the preliminary stages. Even though different lncRNAs seem to have oncogenic or tumor suppressive roles in CC (Figure 2), the exact underlying molecular mechanism of lncRNAs in CC is largely unknown. A number of lncRNAs could be associated with tumorigeneses, invasion, and stemness, leading to therapy resistance in CC. Currently, research is still in the early stages of characterizing the wide range of roles lncRNAs may play in CC pathogenesis. Therefore, more detailed investigation of lncRNAs in CC is needed. This may revolutionize our understanding of CC and result in advances in management, beyond the state of the art. In this regard, molecular tools including ASOs, siRNAs, and CRISPR technology could be used in the future development of RNA-based therapeutics targeting oncogenic lncRNAs.
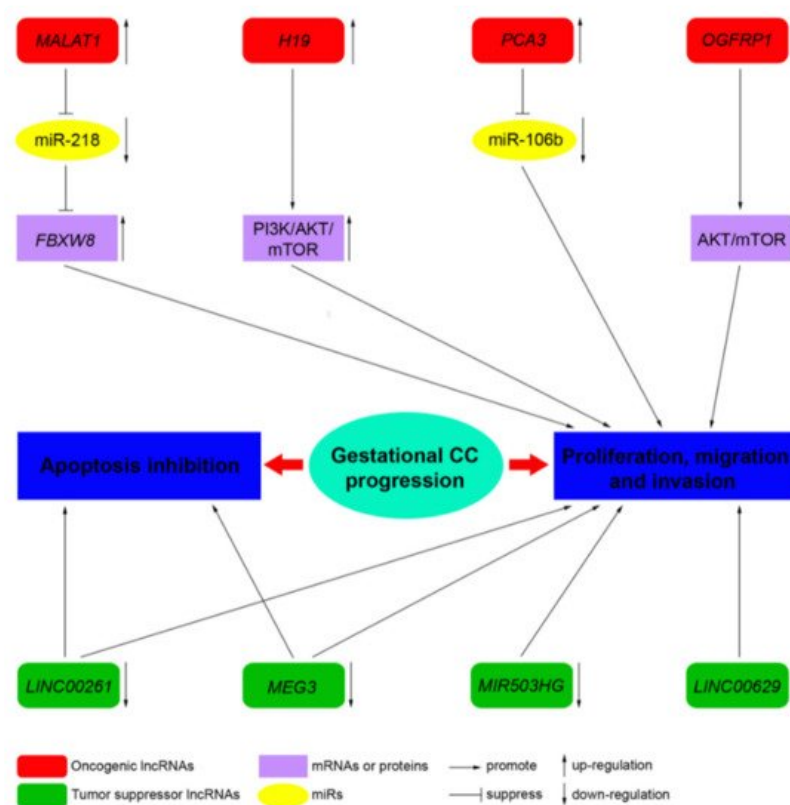

Figure 2. Long non-coding RNAs (lncRNAs) mediate various biological processes to regulate the progression of gestational CC. Certain representative lncRNAs function as oncogenes or tumor suppressors in specific biological processes of gestational CC.

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