THE INVESTIGATION OF THE SKA3 CIRCULAR RNA ROLE IN THE OVARIAN CANCER PROGRESSION

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ABSTRACT

Circular RNAs (circRNAs) are becoming increasingly more recognized in cancer biology as novel biomarkers due to their high stability as well as tissue- and developmental stage-specific expression. Despite massive improvements made in treatments, ovarian cancer (OC) still is considered one of the deadliest types of gynecologic cancers. *SKA3* gene is known for its role in cell proliferation and apoptosis. A circRNA-seq study conducted by our collaborators suggests that circSKA3 is upregulated in the most aggressive subtype of OC, high-grade serous carcinoma (HGSC). Our study showed that circSKA3 levels were higher in HGSC than in tumors with low malignant potential (LMP). Using functional assays, we demonstrated that overexpression of circSKA3 promoted, while the silence of cirSKA3 suppressed, proliferation, migration, and invasion in OVAR3, and CAOV3 cells derived from HGSC. We found that silencing of circSKA3 resulted in an increase in miR-383-5p levels and upregulation of a miR-383-5p target gene, lactate dehydrogenase-A (LDHA). Interestingly, overexpression of miR-383-5p or silencing of LDHA reversed the effects of circSKA3 in cell invasion. These findings suggest that circSKA3 promotes HGSC development by sponging miR-383-5p to induce LDHA expression and that circSKA3 may be used as a diagnostic or therapeutic target for HGSC in the future.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Chapter 3

Chapter 4

LIST OF ABBREVIATIONS

Competing endogenous RNA	CeRNA
Circular RNA	CircRNA
Circular intronic RNAs	ciRNAs
Clear cell carcinoma	CC
Dulbecco's Modified Eagle Medium	DMEM
Empty vector	EV
Endometrioid carcinoma	EC
Epithelial-to-Mesenchymal Transition	EMT
Epithelial Ovarian Cancer	EOC
Extracellular matrix	ECM
Exonic circRNAs	EcircRNAs
Exon – intron circRNAs	EIciRNAs
Fetal bovine serum	FBS
High-grade serous ovarian cancer	HGSC
Lactate dehydrogenase-A	LDHA
Internal ribosome entry site	IRES
Low-grade serous	LGSC
Low malignant potential tumor	LMP
Matrix metalloproteinases	MMPs
Messenger RNA	mRNA
MicroRNAs	miRNA
MicroRNA-383-5p	miR-383-5p
MiRNA response elements	MREs
Mucinous Carcinoma	MC
Nontargeting negative control	NC
Precursor mRNAs	pre-mRNAs
Precursor tRNA	pre – tRNA
Open reading frame	ORF
Ovarian serous carcinomas	OSC

Ovarian surface epithelial cells	OSE
Ovarian cancer	OC
Quantitative PCR	qPCR
RNA-binding proteins	RPBs
RNA-induced silencing complex	RISC
Sarcoglycan zeta	SGCZ
SKA3 circular RNA	circSKA3
Small interfering RNA	SiRNA
Spindle and kinetochore associated complex subunit 3	SKA3
Stratifin	SFN
The 3′ untranslated region	3′ UTR
tRNA intronic circRNAs	tricRNAs

CHAPTER 1

INTRODUCTION

1.1: Ovarian Cancer

1.1.1: An Overview of Ovarian Cancer

Ovarian cancer (OC) is the fifth-largest cause of cancer-related deaths among women (Stewart *et al.,* 2019). The high mortality rate of OC is due to its late detection, a consequence of early nonspecific symptoms such as gastrointestinal or abdominopelvic pains (Kishorbhai & Malaviya, 2021). When upon diagnosis, in most cases, patients are already in the advanced or late stages.

OC has remained a focus area for cancer researchers; however, there are still gaps in providing a fundamental understanding of its carcinogenesis. Based on the origin of the cancer, OC is categorized into three types: epithelial, stromal, and germ cell tumors. Among them, epithelial ovarian cancer (EOC) is the most lethal type which accounts for 90% of OC and is usually known as the "silent cancer" (Feeney *et al.,* 2020). Intriguingly, most ovarian carcinomas do not originate from the ovaries (Wang *et al*., 2015). Therefore, OC should not be considered as a single disorder, but as multiple diseases with varying risk factors, biological behaviors, prognoses, and gene mutations (Desai *et al.,* 2014).

1.1.2: Epithelial Ovarian Cancer

Besides late detection, the high mortality rate of EOC is also attributed to its different subtypes, each has a distinct origin, precursor lesions, and different chemosensitivity and patient outcomes, thus making it hard to treat EOC (Lheureux *et al.,* 2019).

EOC has four different subtypes based on the histopathological characteristics: serous (high grade and low grade), endometrioid, mucinous, and clear cell carcinoma. Among the subtypes, high-grade serous carcinoma (HGSC) is the most common and aggressive type accounting for 70% to 80% of EOC (Stewart *et al*., 2019), while low-grade serous carcinoma

(LGSC), endometrioid, mucinous, and clear cell subtypes account for <5%, 10%, 2–3%, and 10%, respectively (Devouassoux-Shisheboran & Genestie , 2015).

1.1.3: High-Grade Serous Carcinoma

High-grade serous carcinoma (HGSC) accounts for about 70–80% of EOC deaths and has the worst prognosis (Lisio *et al.*, 2019). Patients mostly show their symptoms at an advanced stage (stage VI or distant metastasis), making treatment less effective. Inactivation of the *TP53* gene has been reported in over 80% of the diagnosed cases of HGSC (Kim *et al*., 2012). Moreover, HGSC is marked with mutations in the tumor suppressor genes such as BRCA 1 and 2 (Kim *et al*., 2012). The high-grade tumors can develop *de novo* from the fallopian tubes (Vang *et al.*, 2009). Morphologically, these tumors have solid growth patterns, atypical and large nuclei (Rosen *et al.*, 2009).

1.1.4: Low-Grade Serous Carcinoma

The origin of low-grade serous carcinoma (LGSC) is considered to be serous borderline tumors. Contrary to HGSC, LGSC has fewer *TP53* mutations, less sensitivity to chemotherapy, and a higher survival rate (Vang *et al.*, 2009). Only 4.66% of serous ovarian carcinomas are LGSC, far less common than HGSC (Vang *et al.*, 2009). LGSC exhibits small uniform nuclei and micropapillary growth patterns.

Although LGSC is highly similar to the low malignant potential tumors (LMP), the latter has not the enhanced p53 signaling activity in the LGSC (Bonome *et al.*, 2005). Nevertheless, both LGSC and LMP have frequent mutations in *KRAS* or *BRAF* genes and are less aggressive as compared to HGSC. Ovarian LMP tumors have the potential to become LGSC following the mutation of *KRAS* (an oncogene in the RAS/MAPK pathway that is involved in cell division and differentiation) or attenuation of p53 signaling (Gadducci & Cosio, 2020; Bonome *et al*., 2005).

1.1.5: Endometrioid Carcinoma

Endometrioid carcinoma (EC) is originated from endometriosis and accounts for 10–20% of EOC (Rosen *et al.*, 2009). It is known as an early-stage disease with grade 1 histologic manifestation and has a favorable survival rate compared to serous carcinomas (Rosen *et al.*, 2009). However, if patients are not diagnosed in time, their prognosis will get worse than HGSC as EC advances (Storey *et al.*, 2008).

EC is characterized by frequent mutations in *CTNNB1*, *PIK3CA*, *KRAS*, *ARID1A*, *PTEN*, and *PPP2R1A* genes (Pierson *et al.*, 2020). Mutations in the *CTNNB1* gene, which encodes β-catenin protein critically involved in the development, are rarely seen in other EOC subtypes except for EC (Ovarian Cancers: Evolving Paradigms, 2016). Morphological analysis showed that EC has two growth patterns: cystic and solid (Rosen *et al.*, 2009).

1.1.6: Clear Cell Carcinoma

Like EC, clear cell carcinoma (CCC) also is derived from endometriosis. It accounts for about 3–12.1% of the EOC (Rosen *et al.*, 2009). Early diagnosis of CCC is easy to make due to the presence of enlargement or swelling in the pelvic region (Rosen *et al.*, 2009). Half of the CCC cases have deactivation of a tumor suppressor gene, *ARID1A* (Ovarian Cancers: Evolving Paradigms, 2016). Additionally, mutations in *PIK3CA*, *PPP2R1A*, *PTEN*, *KRAS*, and *TP53* genes are also observed, though they are not as significant as the *ARID1A* gene (Ovarian Cancers: Evolving Paradigms, 2016). The morphological characteristics of CCC are highlighted in its several growth patterns, such as solid, papillary, tubulocystic and clear cytoplasmic due to the presence of intracytoplasmic glycogen (Ovarian Cancers: Evolving Paradigms, 2016).

1.1.7: Mucinous Carcinoma

Mucinous carcinoma (MC) is the most common subtype in women under the age of 40; however, it is the least common type of OC (3%) (Ovarian Cancers: Evolving Paradigms, 2016; Babaier & Ghatage, 2020). The genomic instability of MC is due to the activation of KRAS (the most common type), BRAF, and ERBB2 and the deactivation of tumor suppressor genes such as TP53, CDKN2A, and RNF43 (Babaier & Ghatage, 2020). Although MC is considered one of the EOC subtypes, it has a completely different entity than others. Unlike most EOC tumors that are homogeneous, MC has a heterogeneous cellular composition, in that all of the benign, borderline, and malignant elements are present in the same tumor (Rosen *et al.*, 2009). Moreover, MC has endocervical-like or intestinal-like cell types, or both in some cases. Tumors that have intestinal-like phenotypes are borderline and malignant (Rosen *et al.*, 2009). It is believed that MC is derived from remote sites, such as the gastrointestinal and biliary tracts or pancreas (Ovarian Cancers: Evolving Paradigms, 2016).

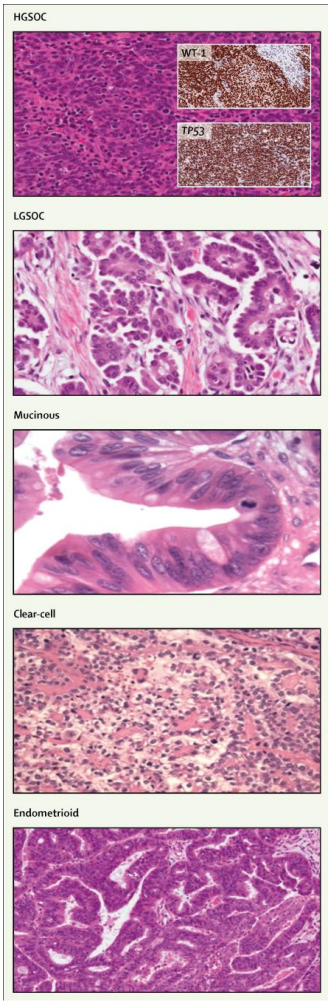

Figure 1.1: The histological features of different subtypes of EOC. From top to bottom, high-grade serous, low-grade serous, mucinous, clear cell, and endometrioid carcinoma. Obtained with permission from (Lheureux *et al.*, 2019) with modifications.

1.1.8: EOC Stages and Grades

EOC is classified into four stages (I–IV) based on cancer progression, and the survival rate is dependent on the stage of cancer (Colombo *et al*., 2006). As the stage increases, the survival rate decreases. Stage I is the least advanced stage where the cancer is in the ovaries and has not spread to other regions. In stage II of the EOC, cancer extends from the ovaries to the pelvic region. During stage III, it passes the pelvic region and reaches the visceral peritoneum. Finally, stage IV is classed as "distant metastasis" as tumors extend to the remote organs. Based on morphology, degree of differentiation, and aggressiveness of cancer cells, EOC is categorized into three grades. The grades of EOC describe how much cancer cells are different in appearance from the normal and healthy cells. With the grade increase, the cancer cells become poorly differentiated, and tumors become more aggressive causing decreased survival rate (Williams *et al*., 2007). Grade 1 refers to the cancer cells that are well-differentiated, resembling normal ovarian cells, and less likely to spread. Grade 2 describes cancer cells that are moderately differentiated, abnormalities in appearance compared to normal cells increase, and tend to grow faster compared to grade 1. Lastly, in grade 3, cells are poorly differentiated, do not look like normal cells, and more likely to spread.

1.1.9: EOC Origins

EOC was previously considered to arise from the ovarian surface epithelial cells (OSE). Cheng *et al.* (2005) supported OSE as the origin of EOC by studying HOX genes. Their study showed that ectopic expression of different variants of HOX genes in mouse OSE cells leads to different EOC subtypes (Cheng *et al.,* 2005). Nevertheless, several studies have recently refuted this theory and showed that each subtype of EOC has different origins, most of which start from outside of the ovaries. The fallopian tube is considered to be the origin of HGSC and serous

borderline tumors for LGSC (Yang-Hartwich *et al.,* 2014). MC origin is traced back to the gastrointestinal tract or cervix, whereas endometriosis is postulated as the origin of CCC and EC (Yang-Hartwich *et al.,* 2014). Yang-Hartwich *et al.* (2014) investigated how extra-ovarian malignant cells migrate to the ovary from their origin and promote the establishment of tumors. Using a mouse model, they showed that during ovulation, chemoattractant chemokines (such as SDF-1) in ovaries increased the migration of ovarian tumor-initiating cells (OTICs) toward the ovaries. Once inside the ovary, the ovarian stroma secretes extracellular matrix (ECM) to help these extra-ovarian malignant cells to adhere and form tumors. However, the recruitment of cancer cells and the formation of tumors in the ovary involve many cell signaling networks.

1.1.10: Risk Factors of EOC

There are various risk factors linked to the increased likelihood of an individual developing EOC. The first risk factor is age, and the risk increases with the advancement of age. EOC affects women as young as 15, and it is deadly to women aged 56 years and above (Desai *et al.,* 2014). The second risk factor is familial history as EOC is considered a genetic disease. Most EOC tumors that are due to a hereditary predisposition are caused by mutations in tumor suppressor genes such as BRCA (*BRCA1* and *BRCA2*) which are involved in DNA repair (Desai *et al.*, 2014). Besides the genetic factors, excessive ovulation (releasing eggs from the ovary by rapturing follicles) is associated with the formation of EOC. After ovulation, ovaries secrete cytokines and growth factors to repair rapture follicles creating an inflammatory status. By increasing ovulation, the repetitive process of rupture and repair in the ovaries leads to a suitable environment for the extra-ovarian pre-malignant and malignant cells to grow in the ovary (Yang-Hartwich *et al.,* 2014). Factors such as multiple pregnancies, late menstrual cycle, and the use of oral

contraceptives can reduce ovulation thereby reducing the risk of EOC (Yang-Hartwich *et al.,* 2014).

1.1.11: Treatment of EOC

Although EOC is one of the deadliest illnesses among women, several interventions have been instituted to treat and prevent the cancer, including early screening. There are three main treatments for EOC: surgery, chemotherapy, and radiotherapy (Desai *et al.,* 2014). Overall, treatment is usually selected based on the tumor's histologic subtype and advancement status. Radiation therapy is rarely used for EOC because of its inefficiency. The most common therapeutic approach is surgery followed by chemotherapy. Current chemotherapies involve platinum-based drugs (e.g., cisplatin and carboplatin) with taxane agents (e.g., paclitaxel) (Ovarian Cancers: Evolving Paradigms, 2016). However, despite the aforementioned treatments, the risk of recurrence and emerging drug resistance in patients, especially those in advanced stages, drive researchers to develop novel therapies (Tarhriz *et al*., 2018).

1.2: Circular RNA

1.2.1: An Overview of Circular RNA

Circular RNAs (CircRNAs) are a class of single-stranded RNAs that are formed through a back-splicing process, in which a downstream 5′ splice site of one exon is joined to an upstream 3′ splice site of another axon via a covalent bond. This feature of circRNAs protects them from exonucleases-mediated degradation, making them highly stable compared to linear mRNAs (Arnaiz *et al.*, 2019). In the past, circRNAs were considered "functionless by-products of aberrant RNA splicing" with no biological function due to their low expression levels (Su *et al.,* 2019; Qu *et al.,* 2015). However, lately, with the help of high-throughput sequencing technology, circRNAs

are found to be abundant in eukaryotes and exert different biological functions by regulating genes involved in various physiological conditions and developmental stages (Su *et al.,* 2019). Furthermore, because circRNAs exist in extracellular body fluids such as plasma, saliva, and exosomes, they are now widely considered to be potential biomarkers or therapeutic targets in human diseases (Vo *et al.,* 2019).

Possession of unique and shared properties, such as high stability, diversity, abundance in the cytoplasm, and conservation among mammalian species permits circRNAs to have a variety of functions (Barrett & Salzman, 2016). They play important roles in regulating gene expression at post-transcriptional and transcriptional levels and are implicated in several pathological processes such as cancer and neural diseases (Wu *et al.,* 2019). CircRNAs are mostly involved in the post-transcriptional process by acting as a microRNA (miRNA) sponge, which is the first and commonly reported function of circRNAs, especially in the context of cancer.

CircRNAs are becoming highly recognized in cancer research as they are involved in regulating the hallmarks of cancer such as sustained angiogenesis, tissue invasion, metastasis, evading apoptosis, and so on (Su *et al.,* 2019). Interestingly, some reports have shown that circRNAs induce resistance to chemotherapy (Su *et al.,* 2019). For example, circPVT1 (from the *PVT1* gene) knockdown suppresses the expression of ATP-binding cassette B1 protein, which is resistant to doxorubicin and cisplatin, and eventually reduces chemotherapy resistance (Su *et al.,* 2019).

Although circRNAs have high stability compared to linear RNAs, there are some hypotheses that circRNAs can be degraded by endonucleases such as RNase L or transported out of cells via exosomes (extracellular vesicles that carry nucleic acids) for degradation (Zhao *et al.,* 2019). More studies are needed to understand the mechanism of circRNA degradation.

1.2.2: Biogenesis of CircRNAs

There are four different types of circRNAs based on their structures and formation. First, exonic circRNAs (ecircRNAs), which consist of one or more exons, are the most common type accounting for 80% of circRNAs and are localized in the cytoplasm (Zhao *et al.*, 2019). Second, exon-intron circRNAs (EIciRNAs) are derived from both exons and introns. Third, circular intronic RNAs (ciRNAs) contain only introns. Both EIciRNAs and ciRNAs are located in the nucleus. Lastly, tRNA intronic circRNAs (tricRNAs) also contain only introns; however, they are originated from precursor tRNA (pre-tRNA) and found in archaea and drosophila (Zhao *et al.*, 2019).

Dragomir & Calin (2018) argued that the expression of circRNAs varies depending on cell types and ontogenesis stages, implying that circRNA formation is a regulated process. Like linear RNAs, circRNAs are also derived from the precursor mRNAs (pre-mRNAs). CircRNAs can be generated through several models, including a lariat-driven circularization (exon skipping) model, intron pairing-driven circularizing (direct back splicing) model, and RNA-binding proteins (RBPs)-mediated circularization model.

In the lariat-driven circularization model, during the pre-mRNA splicing process, a lariat containing both exons and introns is formed when the upstream acceptor and downstream splice donor get close. Then introns get spliced, and exons join each other via a covalent bond to form the ecircRNA (Zhao *et al.*, 2019). However, in some cases, the introns are not removed which results in the formation of EIcircRNAs (Geng *et al.*, 2020). When a 7 nt GU-rich sequence is present at the 5′ splicing site and an 11 nt C-rich motif at the 3′ site, it can promote the formation of a lariat only containing introns which eventually leads to the formation of ciRNAs (Su *et al.*, 2019).

The intron pairing-driven circularizing model happens when upstream splice acceptor and downstream donor have inverted complementary sequences, such as ALU sequence, and join via complementary base pairing to form ecircRNAs or EIcircRNAs. For the circRNAs to form through this model, each acceptor and donor should have at least 100 nt of ALU complementary sequence (Huang *et al.*, 2020).

The involvement of RBP directs the third mechanism that leads to circRNA formation. Similar to the exon skipping model, the upstream and downstream splice sites get close together and RBPs are responsible for this proximity by forming a bridge flanking the splice sites and promoting back splicing (Zhao *et al.*, 2019). Finally, in the formation of the tricRNAs, after the pre-tRNA is spliced by enzymes to form tRNA, circRNA is formed from the remaining introns of pre-tRNA (Geng *et al.*, 2020). So far, the step-by-step formation of circRNAs is still not well understood.

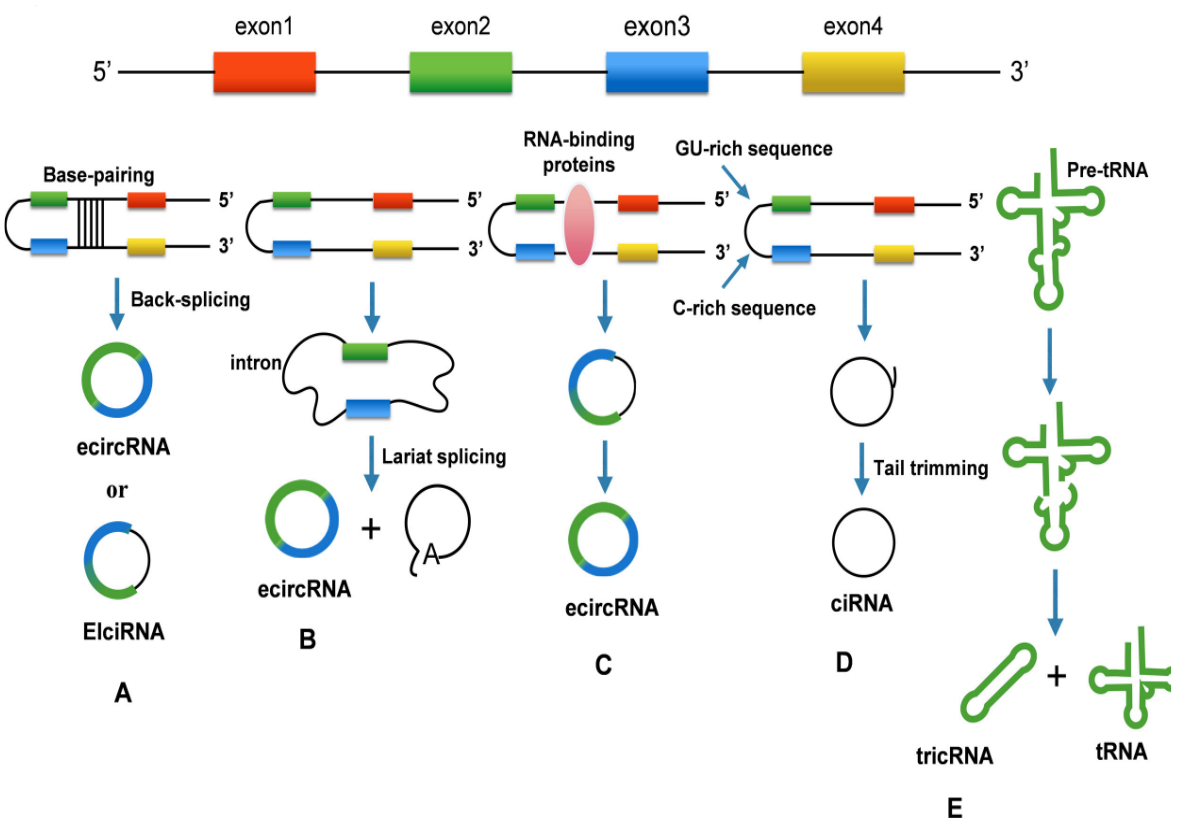

Figure 1.2: The biogenesis and different types of circRNAs. CircRNAs can be generated through multiple mechanisms. A) an intron pairing-driven circularizing (direct back splicing) model: the inverted complementary sequences in upstream acceptor and downstream donor join via complementary base pairing, leading to the formation of ecircRNA or ElciRNA (if the introns are not removed). B) a lariat-driven circularization (exon skipping) model: a lariat is formed during pre-RNA splicing and then the spliceosome removes introns to generate ecircRNAs. C) an RBP-driven model: some RBPs can cause the acceptor and donor to get close and promote circularization and result in the formation of ecircRNAs. D) The formation of ciRNAs requires the presence of 7 nt GU-rich sequences and 11 nt C-rich motifs. E) tricRNAs are formed from the leftover introns of pre-tRNA after splicing. Obtained with permission from (Lei *et al.*, 2018).

1.2.3: Functional Mechanisms of CircRNAs

In general, four mechanisms have been proposed for the actions of circRNAs. First, some circRNAs have miRNA response elements (MREs, also known as binding sites). Therefore, they can act as miRNA sponges to inhibit their expression and thus indirectly regulate the downstream targets of miRNAs.

The second mechanism is to interact with proteins. This interaction can be bidirectional (Das *et al.*, 2021). CircRNAs can act as either protein decoys/sponges or scaffolds. As protein sponges, they can bind to proteins and modulate their interaction with target proteins or mRNAs. As protein scaffolds, they can either recruit proteins to specific locations or facilitate contact of proteins to influence biological processes (Zhao *et al.,* 2019). The interaction between proteins and circRNAs can occur by harboring RNA-binding protein sites or the unique tertiary structure of the circRNA.

Another crucial function of circRNAs is the translation of proteins. Until recently, circRNAs were considered noncoding genes; however, recent studies have shown that some circRNAs have a translational ability. They exert their effects by encoding functional proteins with around 146–344 amino acids, which are mostly found in cancer cells or myoblasts (Zhao *et al.,* 2019). An internal ribosome entry site (IRES) and an open reading frame (ORF) are needed for circRNAs to encode proteins (Zhao *et al.,* 2019).

Finally, circRNAs can also regulate the expression of their host genes. This function is mainly reported in EIciRNAs and ciRNAs that are in the nucleus (Zhao *et al.,* 2019). By binding to RNA polymerase II, circRNAs can regulate the transcription levels of their linear genes. So far, EIciRNAs and ciRNAs are reported to enhance the linear RNA transcription levels (Lei *et al.,* 2018). Moreover, some ecircRNAs also regulate their host gene expression by localizing in the

nucleus. CircRNAs may compete with their linear mRNA for the same exon, and this will lead to the suppression of linear mRNA expression (Lei *et al.,* 2018).

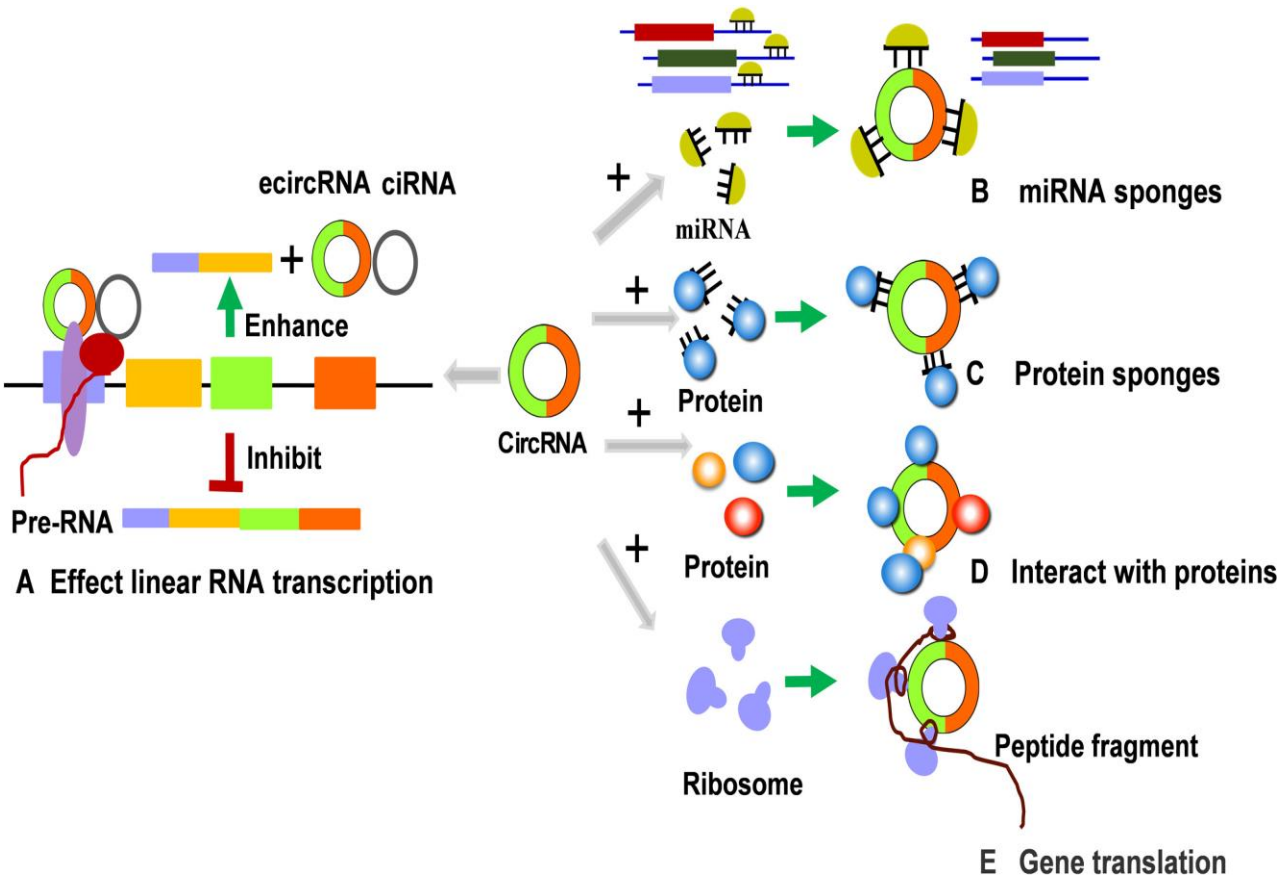

Figure 1.3: The mechanisms of circRNA actions. A) Regulation of their linear gene. EcircRNAs can suppress or enhance the transcription of linear mRNA depending on whether they have common exons. For ciRNAs and EIcircRNAs, transcription levels of linear mRNAs are enhanced. B) CircRNAs can act as miRNA sponges to inhibit their function. C) CircRNAs can also be protein sponges and suppress their function. D) CircRNAs can interact with proteins to regulate biological processes. E) CircRNAs exert their effect through translating functional proteins or peptides. Obtained with permission from (Lei *et al.*, 2018).

1.3 SKA3 Gene

1.3.1: An Overview of SKA3 Gene

The Spindle and kinetochore-associated complex subunit 3 (SKA3) belongs to the SKA family and plays a role in chromosome separation and cell division (Zhong *et al*., 2021). Together with SKA1 and SKA2, SKA3 assists chromosomes to separate during anaphase by keeping kinetochores attached to the microtubules (Abad *et al.*, 2016). So, SKA3 is required for a timely mitotic progression (Liu *et al.*, 2020). Abad *et al.* (2016) showed that without the SKA3 C-terminal domain, the binding efficiency of kinetochores to the microtubules was reduced causing a delayed separation of chromosomes.

SKA3 is located on chromosome 13q12.11. Though the *SKA3* gene is known for its function in normal cell proliferation and apoptosis (Gao *et al*., 2020), it is strongly associated with the growth of many cancers such as breast, lung, and liver cancers. The role and mechanism of SKA3 in OC are still unknown.

Nine circular RNAs are derived from the SKA3 gene. Previous reports of different SKA3 circular RNA (circSKA3) isoforms showed that they have tumor-promoting effects in cancers such as medulloblastoma and breast cancer, by increasing tumor cell proliferation, migration, and invasion capabilities through sponging miRNAs or binding with proteins depending on the cancer type.

1.3.2: CircSKA3

In breast cancer, circSKA3 (has_circ_0000467) with 412 base pairs (bp), by binding to Tks5 and integrin β1 proteins, induces the formation of membrane protrusions (Invadopodium) that subsequently promotes cancer invasion (Du *et al.*, 2020). Interestingly, Du *et al*. (2021) showed that circSKA3 was transferred between cancer cells via exosomes and enhanced the

invasive property of neighbor breast cancer cells. CircSKA3 has also been shown to function as a miRNA sponge in many cancers. Based on the previously published articles, circSKA3 sponges miR-326-3p, miR-382-5p, miR-1, miR-383-5p, and miR-326 in gastric cancer, colorectal cancer, glioblastoma and medulloblastoma, respectively (Mo *et al.*, 2020; Xie & Pan, 2021; Zhou *et al.*, 2021; Wang *et al.*, 2020; Zhao *et al.*, 2021).

Other than cancer, circSKA3 has a controversial role in heart diseases such as ischemic stroke and cardiotoxicity. So far, sponging miRNAs is a reported function of circSKA3 in these disorders (Xu *et al.*, 2022; Li *et al*., 2021). CircSKA3 exerts its effects by binding to proteins or sponging miRNAs depending on the disease.

1.4: MicroRNAs

miRNAs are short single-stranded non-coding RNAs (19–25 nt) known as "powerful regulators" involved in the regulation of the post-transcriptional expression of their target genes (O'Brien *et al.*, 2018). In general, miRNAs inhibit the expression of target genes via mRNA degradation or suppression of their translation by interacting with the 3′ untranslated region (3′ UTR) of target genes. After interacting with an RNA-induced silencing complex (RISC) containing Ago-2 protein, the mature miRNA will bind to target mRNA at MREs located at 3′ UTR of the target gene. On rare occasions, if the binding of a miRNA to the target gene is fully complementary, it will induce endonuclease activity of Ago-2 to cleave mRNA for degradation. In most cases, when the binding of a miRNA to the target gene is not fully complementary, Ago-2 will not be activated but act as a mediator of RNA interference (O'Brien *et al.*, 2018).

Normally, gene regulation by miRNAs is essential for biological processes such as cell cycle, development, differentiation, and so on (Acunzo *et al.* ,2015). However aberrant regulation

of miRNAs leads to diseases such as cancer. Many factors induce abnormal expression or function of miRNAs, including circRNAs.

1.4.1: MiRNAs and CircRNAs

The most common role of circRNAs is acting as a miRNA sponge, as they can sequester miRNAs from their target genes by binding to miRNAs and interrupting their interactions. CircRNAs can act as miRNA sponges when they have a great number of miRNA binding sites, or they have a high abundance. By acting as a competing endogenous RNA (ceRNA), circRNAs can bind to miRNAs and regulate their functions. This results in indirect regulation of genes that modulate processes such as proliferation, migration, and invasion (Bai *et al.,* 2020). Having a complementary sequence to the seeding region of miRNA (MREs), circRNAs can bind to miRNAs as a sponge. This will reduce the availability of miRNAs and release the inhibitory effect on their target mRNAs, resulting in upregulated translation of target genes (Kristensen *et al.*, 2019; He *et al.*, 2021). It is hypothesized that circRNAs can be degraded while interacting with miRNAs during the Ago-2-facilitated cleavage process (Kristensen *et al.,* 2019). However, circRNAs may not always act as miRNA sponges even though they might have a small number of miRNA binding sites (He *et al.*, 2021).

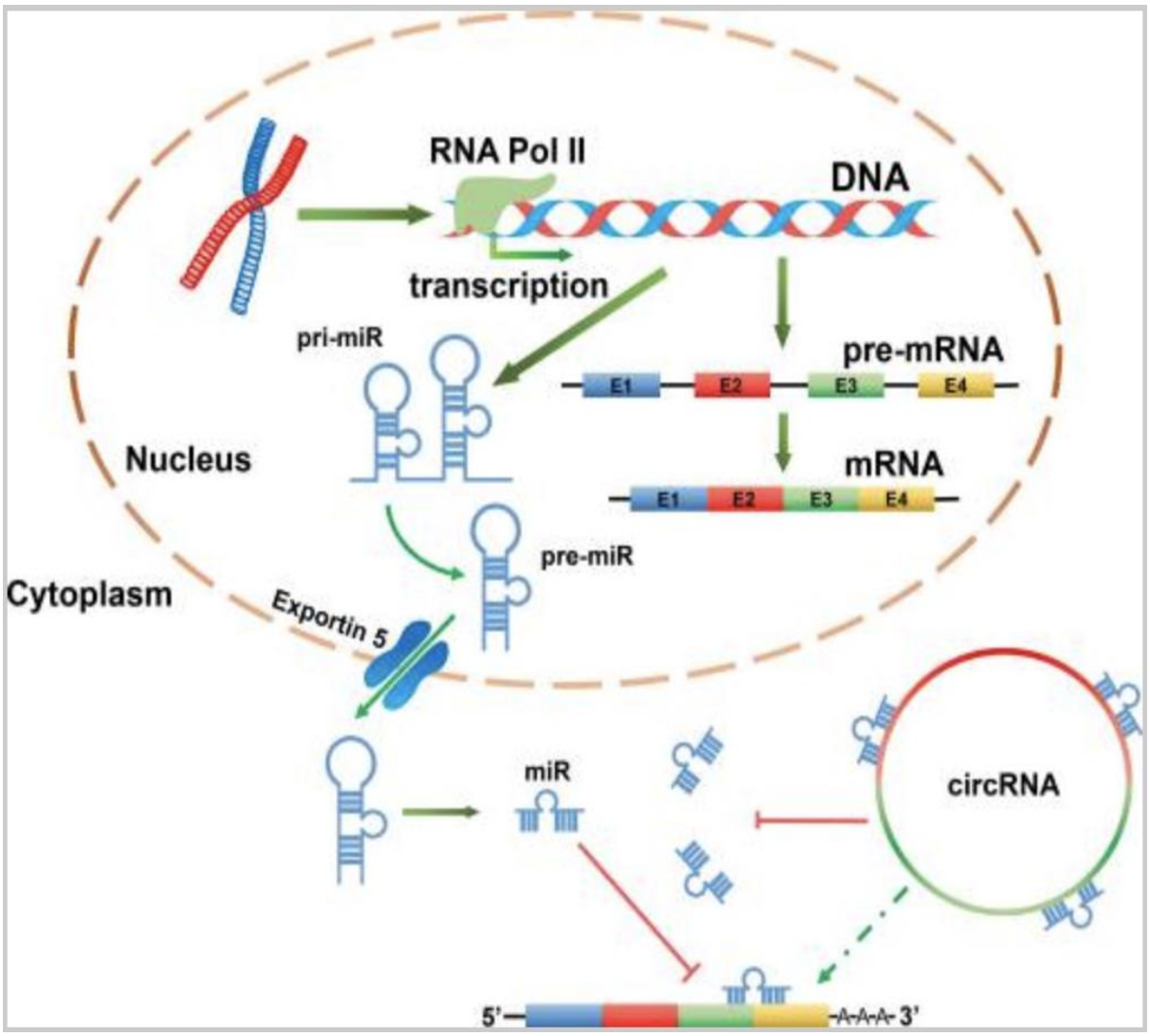

Figure 1.4: Interaction of circRNAs with miRNAs. After miRNAs get mature in the cytoplasm, they may interact with circRNAs and their mRNA targets. CircRNAs can compete with mRNA targets for miRNAs depending on their abundance and whether they have miRNA response elements (MREs). Once circRNAs bind to miRNAs, they can sequester them from their targets and indirectly upregulate target gene expression. Obtained with permission from (Han *et al.*, 2017).

1.5: Lactate Dehydrogenase-A

Glycolysis is a process that without requiring oxygen, produces energy for tissues by breaking down glucose. One of the vital enzymes of glycolysis is lactate dehydrogenase-A (LDHA) whose main role is to catalyze the conversion of pyruvate into lactate. Mostly, it is located in the cytoplasm and has 332 amino acids.

The activation status and over-expression profile of LDHA has been associated with many different cancers including OC (Feng *et al.,* 2018). Upregulation of LDHA causes cancer cells to switch to aerobic glycolysis which contributes to their high survival rate (Jin *et al.*, 2017). LDHA has been involved and contributed to different aspects of cancer such as proliferation, invasion, migration, and so on. That is why in many clinical trials, LDHA has become a favorite target for diagnosis and treatment *(*Feng *et al.,* 2018). Accumulating evidence suggests that LDHA has a significant impact on the invasion and migration aspect of cancer usually by producing high lactate output (Feng *et al.,* 2018).

In the EOC, Han *et al.,* (2017) stated that over-expression of LDHA may be due to the suppression of miR-383. Their study provided strong evidence about the regulatory relationship between miR-383 and LDHA. They reported that over-expression of LDHA impaired the suppressing effect of miR-383, while knocking down LDHA reversed the miR-383 inhibitor's protumoral effect on the proliferation and invasion of malignant cells.

1.6: Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is a cellular event in which cells lose their epithelial characteristics and acquire mesenchymal features that enhances their migrative and invasive abilities. This process is essential for normal embryonic development, tissue regeneration,

organ fibrosis, and wound healing (Lu & Kang, 2019; Serrano-Gomez *et al.,* 2016). However, a defect in the process can cause diseases such as cancer and lead to chemo and immune resistance (Jiang *et al.,* 2021).

Loss of E-cadherin expression (epithelial marker) and induction of N-cadherin, Snail, Slug, Vimentin, Fibronectin, and Twist (mesenchymal markers) are hallmarks of the EMT. E-cadherin marker is considered to be the hallmark of epithelial cells (Son and Moon *et al.,* 2010). It is encoded by the CDH1 gene and leads to cell-to-cell adhesion. Downregulation of E-cadherin causes cells to be free from their restrictive cell-to-cell contacts. While losing E-cadherin, cancer cells substitute for N-cadherin to help them get separated from their neighboring cells and migrate freely to the new location (Liu *et al.,* 2014). Encoded by the CDH2 gene, N-cadherin can be a sign of ongoing EMT (Loh *et al.,* 2019). Snail and Slug are belonging to the SNAIL superfamily of zinc-finger transcription factors that can stimulate EMT by promoting β-catenin–LEF-1 activity. This promotion leads to the initiation of EMT (Medici *et al.,* 2008). During EMT, when cancer cells are migrating and squeezing through narrow spaces to reach their destination, they face mechanical stress. To overcome these stresses, Vimentin expression is upregulated in cells (Usman *et al.,* 2021). Vimentin is a type III intermediate filament protein present in the mesenchymal cells with structural support as the main role; It supports the positioning and integrity of organelles by the viscoelastic framework.

For a malignant progression to occur, cell invasion is an essential step in which EMT plays an important role in developing invasive potential (Son and Moon, 2010). Matrix metalloproteinases (MMPs) are a group of proteins that are responsible for the degradation of ECM. By degrading ECM, they will stimulate EMT, and EMT in its turn produces more MMPs which induce cell invasion (Son and Moon, 2010).

Many circRNAs are involved in cancer progression by regulating the EMT process. If circRNAs act as tumor-promoting genes, they can downregulate epithelial markers and upregulate mesenchymal markers. On the other hand, circRNAs may inversely regulate epithelial and mesenchymal markers if they function as tumor suppressors. CircRNAs usually affect EMT by regulating other genes such as miRNAs. For example, circNUP214 upregulates ZEB2 which is a transcription factor that regulates EMT by sponging miR-145, thereby promoting EMT in papillary thyroid cancer (Jiang *et al.,* 2021).

1.7: Rationale, Hypothesis, and Objectives of Present Study

Understanding the mechanisms of OC progression and seeking new treatments remain imperative among cancer researchers. OC is the most aggressive gynecologic cancer and one of the leading causes of death among women (Yang *et al.,* 2020). Due to their non-obvious early symptoms, OC is typically diagnosed at late stages resulting in low survival rates (Yang *et al.,* 2020). circRNAs possess a variety of functions and are abnormally expressed in various OC tissues (Yang *et al.,* 2020), suggesting that they can be used as diagnostic markers and/or therapeutic targets.

Of the nine circSKA3 isoforms, hsa_circ_0000467 is the smallest circSKA3 with only 412 bp. Based on circRNA-seq data provided by Dr. Burton Yang's laboratory, this circSKA3 isoform is one of the significantly overexpressed circRNAs in HGSC tumors. As such, we hypothesized that circSKA3 promotes tumor growth in OC. The objectives of the project were (1) to study the function of circSKA3 in OC, and (2) to investigate the mechanism of circSKA3 in OC progression.

CHAPTER 2

MATERIALS AND METHODS

2.1: Ovarian Cell lines and Cell Culture

Cells were maintained in an incubator at 37 °C with 95% humidity and 5% CO_2. As models of high-grade serous carcinoma (HGSC): OVCAR3, HEY, and CAOV3 cells were maintained in DMEM media (Thermo Scientific cat # 11965118). The other three different epithelial ovarian cancer cell lines were as follows: TOV-112D cells as endometrioid carcinoma model was maintained in MCDB105/M199 media (Sigma Aldrich cat # M6395/cat # M5017)). SKOV3.ip1 and ES-2 cells as endometrioid and mixed feature of different subtypes respectively were maintained in McCoy's 5A media (Sigma Aldrich cat # M4892). All the media were supplemented with 10% fetal bovine serum (FBS) except for CAOV3 cells, 20% of FBS was used. SKOV3.ip1 and HEY cells were kindly provided by Dr. Mien-Chie Hung (University of Texas M.D. Anderson Cancer Center, Huston, Texas) and Dr. Theodore Brown (Mount Sinai Hospital, Toronto, Canada) respectively. TOV – 112D, OVCAR3, and CAOV3 were purchased from American Type Culture Collection (Manassas, VA, USA).

2.2: Transient Transfection

24 hours after seeding EOC cells into the 6 well plates:

For the overexpression of circSKA3, 4 µg of plasmids (circSKA3 and empty vector (EV)) with 5 µl of P3000 were mixed in 250 µl of Opti-MEM (Thermo Scientific). In the second tube, 3 µl of Lipofectamine 3000 (Life Technologies) was added to 250 µl of Opti-MEM and incubated for 5 minutes at room temperature. The two mixtures were combined and incubated for 20 minutes at room temperature. The 500 µl of the final mixture was added per well to the EOC cells and incubated for 5 hours. After 5 hours, the mixture was replaced with a growth culture medium. Similarly, for the co-transfections of OVCAR3 cells, lipofectamine 3000 was used with

concentrations of 4 μg/well for circSKA3-expressing or EV plasmids, 100 nM/well for miR-383-5p mimics or NC, and 200 nM/well for si-LDHA or NC.

For the silencing of circSKA3, per well, 20 nM of siRNAs (si-circSKA3 and nontargeting negative control (NC)) was added to 120 μl of Opti-MEM. In the second tube, 2 μl of Lipofectamine RNAiMAX (Life Technologies) was added to 120 μl of Opti-MEM and incubated for 5 minutes. The two mixtures were combined and incubated for 20 minutes at room temperature. The 240 μl of final mixture with 360 μl of Opti-MEM was added to the EOC cells and incubated for 5 hours. Finally, the mixture was replaced with a growth culture medium.

The circSKA3 overexpression and EV plasmids were kindly provided by Dr. Burton Yang (Du *et al.,* 2020) siRNAs for circSKA3 (Du *et al.,* 2020), LDHA (Le *et al.,* 2010), NC, and miR-383-5p mimics were purchased from GenePharma Co. Their sequences are listed in Table 1.

Table 1: siRNAs, and miRNA n sequences

Name	Sequence
si-circSKA3	5’ GAGGAAUAAUAAAAGUACATT 3’ 3’ TTCUCCUUAUUAUUUUCAUGU 5’
S i-LDHA	5’ GGCAAAGACUAUAAUGUAATT 3’ 3’ TTCCGUUUCUGAUAUUACAUU5’
miR-383-5p mimics	5’ AGAUCAGAAGGUGAUUGUGGCU 3’ 3’ UCUAGUCUUCCACUAACACCGA 5’

2.3: RNA Extraction, Reverse Transcription, and Real-time PCR

Total RNA was extracted from cells and ovarian tumor tissue samples using the GENEzol TriRNA Pure kit (FroggaBio) based on the manufacturer's suggested protocol.

To measure the level of circRNA and other genes, 2 μg of RNA was subjected to reverse transcription using iScript RT kits following the manufacturer's protocol. The reverse transcription step was performed with 5X reaction buffer (which contained a mixture of oligo(dt) and random hexamer primers) and iScript Reverse transcriptase in a total volume of 40 μL. Then, quantitative PCR (qPCR) was carried out using BlasTaq 2xqPCR Master Mix (ABM), 10 μM of forward and reverse primers in 20 μl volumes using the following thermocycling conditions: annealing temperature of 60° C with 35 cycles.

To measure miRNA level, 2 μg of RNA was subjected to reverse transcription using NCode miRNA first-strand cDNA synthesis kit (Invitrogen) following the manufacturer's suggested protocol. Then, qPCR was carried out using BlasTaq 2xqPCR Master Mix (ABM) with miRNA-specific primers (Table 2) and a universal qPCR primer from the Ncode Kit. Thermocycling conditions were the same as mentioned above.

Relative expressions of circRNA, mRNAs, and miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method, in which the target gene was first normalized to an endogenous reference gene and then expressed as the fold change relative to the control. For circSKA3 and miR-383-5p, U6 and for the rest of the genes, β-actin were used as internal controls. The sequences of primers are listed in Table 2.

Table 2: Primer sequences for qPCR	
Primers	Sequence
circSKA3	5’ ACACAATGGGACTTAAAAATGCGA3’ 5’ ACAGATCATCTTTCACATCAGTCT 3’
N-cadherin	5’ AGCAAGCCGCTCTACACCAT 3’ 5’ TGCGCAGTGCTTTCTCTGTC 3’
E-cadherin	5’ GCCAAGCAGCAGTACATTCTACACG 3’ 5’ GCTGTTCTTCACGTGCTCAAAATCC 3’
Snail	5’ CCTCCCTGTCAGATGAGGAC 3’ 5’ CCAGGCTGAGGTATTCCTTG 3’
Slug	5’ ATGAGGAATCTGGCTGCTGT 3’ 5’ CAGGAGAAAATGCCTTTGGA 3’
Vimentin	5’ GAGAACTTTGCCGTTGAAGC 3’ 5’ GCTTCCTGTAGGTGGCAATC 3’
c- Myc	5’ AAACACAAACTTGAACAGCTAC 3’ 5’ ATTTGAGGCAGTTTACATTATGG 3’
LDHA	5’ CACCAAAGATTGTCTCTGGCA 3’ 5’ AAGATGTTCACGTTACGCTGG 3’
SFN	5’ TCCACTACGAGATCGCCAACAG 3’ 5’ GTGTCAGGTTGTCTCGCAGCA 3’
SKA3	5’ CAGATCCCTCTTCACCTACGA 3’ 5’ TCAACGTTTAAAGGGGGACA 3’
miR-383-5p specific primer	5’ AGATCAGAAGGTGATTGTGGCT 3’
β-actin	5’ AAACTGGAACGGTGAAGGTG 3’ 5’ AGAGAAGTGGGGTGGCTTTT 3’
U6	5’ GTGCTCGCTTCGGCAGCACATA 3’ 5’ TGGAACGCTTCACGAATTTGCG 3’

2.4: Proliferation Assay

Proliferation assays were carried out 48 hours after transient transfection. OVCAR3 and CAOV3 cells were seeded into 96-well plates at the density of 3 × 10^3 cells/well and 8 × 10^3 cells/well respectively. Cell proliferation assays were performed using an imaging system called IncuCyte S3 (Essen Bioscience). The scanning for the cells was 4 hours later after the seeding (giving time for the cells to adhere and settle down on the plate) and then images were taken every 2 hours. The media was changed after 2 days to make sure of cells were healthy. With confluency as the parameter, proliferation curves were generated using Incucyte proliferation analysis.

2.5: Wound Healing Assay

For the wound healing assays, 48 hours after transient transfection, OVCAR3 cells at the density of 25.0 × 10^3 cells/well and CAOV3 cells at the density of 35.0 × 10^3 cells/well were seeded into a 96-well plate. 24 hours after seeding them into 96 well plates, a wound was made using the WoundMaker tool (a 96-pin mechanical device). Immediately after making the wound, first cells were washed with DMEM without FBS and then DEMEM with 1% FBS was added. The images were taken every 2 hours using an imaging system called IncuCyte S3 (Essen Bioscience). Migration curves were generated using Incucyte scratch wound analysis with wound confluency as the parameter.

2.6: Transwell Invasion Assay

Transwell invasion assays were performed 48 hours after transient transfection, OVCAR3 cells with a density of 10000 cells/100 µl of serum-free media and CAOV3 cells with a density of 20000 cells/100 µl of serum-free media were seeded into transwell inserts which were pre-coated with growth-factor-reduced Matrigel (BD Biosciences). 500 µl of DMEM with 10% FBS was added into the lower Transwell chamber. After 24 hours of incubation, they were fixed and stained for analysis using Harleco Hemacolor Staining Kit (EMD Chemicals, Gibbstown, NJ). Invaded cell numbers were counted using an automated quantification plugin for ImageJ (O'Brien *et al.*, 2018).

2.7: Western Blot

After washing cells with 1XPBS pH 7.4, proteins were extracted using RIPA lysis buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 10 mm NaF, 0.1% SDS, 1% Nonidet P-40, and 1× protease inhibitor cocktail). After incubating the cell lysates on ice for 20 minutes, they were centrifuged at 12,000×rpm for 10 minutes at 4°C. Using Pierce BCA Protein Assay Kit, the total protein was quantified, and equal amounts of protein (50 µg) were separated via 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, the cell lysates were mixed with a 5X SDS sample buffer (0.25 M Tris-HCl pH 6.8, 0.25% bromophenol blue, 0.5 M DTT, 50% glycerol, 10% SDS) and incubated for 10 minutes at 95°C. Then, proteins were transferred to a polyvinylidene difluoride membrane (PVDF) for one hour. After being blocked with 5% fat-free milk for one hour, the membrane was probed with primary antibodies (Table 3) overnight. After washing the membranes with TBST buffer three times for 10 minutes, they were probed with secondary HRP-coupled antibodies (Table 3) for 1 hour. Proteins were visualized with enhanced

chemiluminescence reagents HRP Substrate (Millipore or Bio-Rad). β-actin was used as the internal control.

Table 3: The list of Primary and Secondary antibodies for Western blot

Name	Company/Catalogue #	Secondary Antibodies	Dilution of Primary Antibodies	Dilution of Secondary Antibodies
Snail	Cell signaling/3879S	Rabbit	1:100	1:4000
Slug	Cell signaling/9585S	Rabbit	1:100	1:4000
Vimentin	Cell signaling/3390S	Mouse	1:500	1:5000
β-actin	Cell signaling/3700S	Mouse	1:1000	1:5000

2.8: Human Epithelial Ovarian Cancer Samples

Human epithelial ovarian cancer samples were kindly provided by Dr. Barbara Vanderhyden, (University of Ottawa, Ottawa Hospital Research Institute, Center for Cancer Therapeutics, Ottawa, ON). The samples were divided into two groups: high-grade serous carcinoma (HGSC) and tumors of low malignant potential (LMP). LMPs are tumors that have proliferative features while lacking stromal invasion (Massad *et al.*, 1991). So, LMP is less aggressive than HGSC (Ouellet *et al.*, 2005).

2.9: Statistical Analysis

GraphPad Prism 8.0 software was used for statistical analysis and making graphs. Significance was defined as $p<0.05$ and results are expressed as mean ±SEM of replicate wells in one experiment. Each experiment was performed two times with different cell preparation and transfection to ensure consistency of the findings. Using Student's t test, comparison between two groups were analyzed. In addition, One-way ANOVA and Two-way ANOVA which both followed by multiple comparisons test were used to analyze multiple groups.

CHAPTER 3

RESULTS

3.1 Expression level of circSKA3 in ovarian tumor samples and cell lines

The first step was to validate aforesaid circRNA-seq data and to examine the circSKA3 role in EOC. Therefore, the expression level of circSKA3 was assessed in EOC tissue samples obtained from the Ottawa Ovarian Cancer Tissue (Supplementary Table S6.1) by qRT-PCR. As demonstrated in Fig 3.1A, circSKA3 levels were upregulated in the HGSC tumors compared to LMP tumors.

Then, circSKA3 levels were compared among several cell lines of EOC. Based on figure 3.1B, CAOV3 cells had the highest expression of circSKA3, while ES-2 had the lowest circSKA3 levels. The expression level of circSKA3 decreases in the order of CAOV3, TOV-112D, HEY, OVCAR3, SKOV3.ip1, and finally ES-2, respectively.

Among them, OVCAR3, HEY, and CAOV3 cells are representing the HGSC model. SKOV3.ip1 and TOV-112D are derived from EC and ES-2 is mixed features of different subtypes. Because of our interest in HGSC as the most aggressive subtype of EOC (Lisio *et al.*, 2019), the decision was made to work with OVCAR3 and CAOV3 cell lines in this project. They were chosen because OVCAR3 cells had the lowest endogenous expression level and CAOV3 cells had the highest endogenous expression level of circSKA3 among HGSC models.

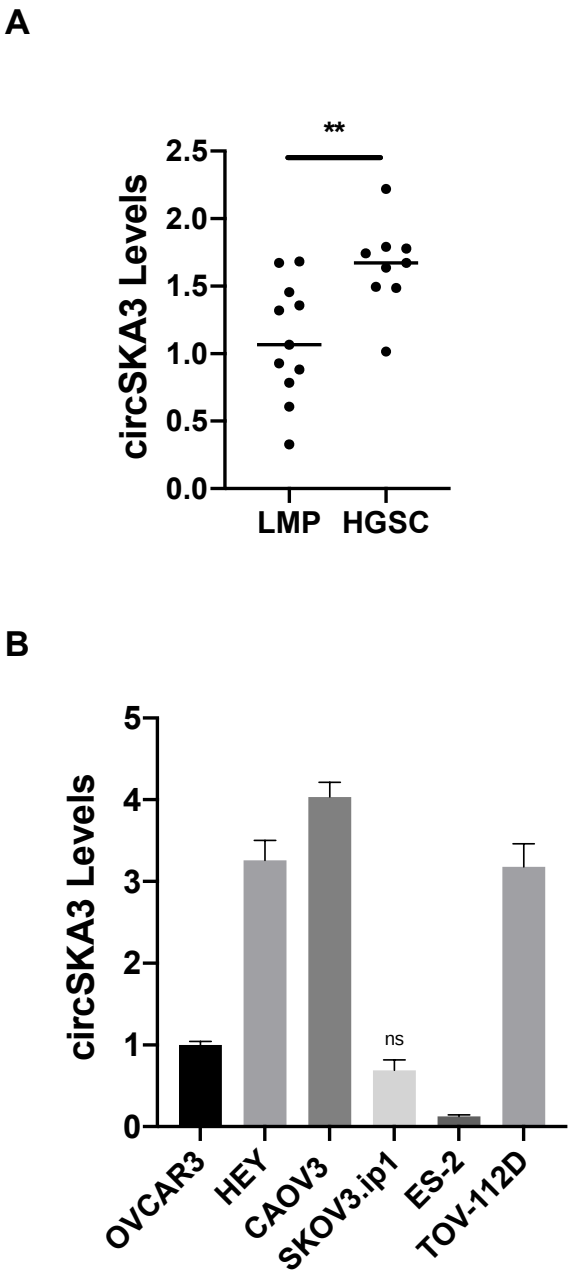

Figure 3.1. Endogenous expression level of circSKA3 in six different cell lines of EOC and tumor tissue samples. A) mRNA expression levels of circSKA3 were significantly upregulated in high-grade serous ovarian tumors (n = 9) as compared to the low malignancy potential tumors (n = 11). B) The expression level of circSKA3 in six different cell lines of EOC. All results are expressed as mean ± SEM (n=3).

3.2 Validation of overexpression and silencing of circSKA3 in HGSC cells

Before performing loss and gain of function experiments, it was needed to validate the expression of circSKA3 after transient transfection with circSKA3-expressing and EV plasmids and lastly siRNAs (si-circSKA3 and NC) since all the experiments in the project were performed with transient transfection. OVCAR3 and CAOV3 cells were transiently transfected with 4 μg /well of plasmids (circSKA3 and EV) and incubated for 48 hours. qRT-PCR results indicated circSKA3 levels in OVCAR3 cells overexpressed with circSKA3 were higher compared to EV transfected cells by about 250-fold (Fig 3.2A). As for CAOV3 overexpressed circSKA3 cells, they showed a 150-fold change higher in circSKA3 levels compared to the EV group (Fig 3.2B).

For silencing of circSKA3, 20nM of siRNAs targeting the junction site of circSKA3 was used. Again after 48 hours, both OVCAR3 and CAOV3 cells transfected with si-circSKA3 showed lower circSKA3 levels about 0.6- and 0.4-fold change compared to NC transfected cells respectively (Fig 3.2 C-D).

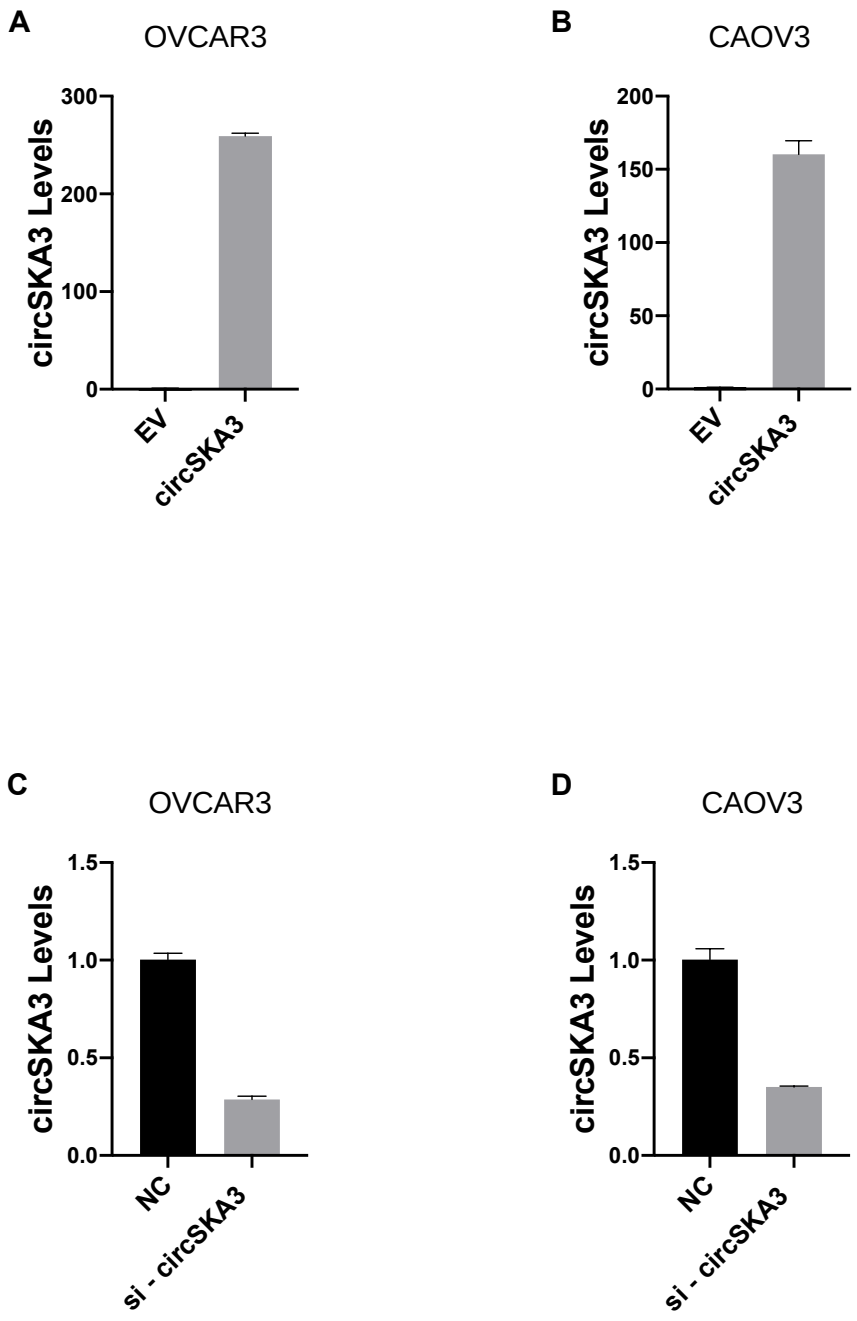

Figure 3.2. Validation of overexpression and silencing of circSKA3 in HGSC cells after transient transfection. The overexpression of circSKA3 in OVCAR3 (A) and CAOV3 (B) cells transfected with circSKA3-expressing, and EV plasmids were confirmed by qRT-PCR. Reduction in circSKA3 levels in both OVCAR3 (C) and CAOV3 (D) cells transfected with si-circSKA3 compared to the NC transfected cells were also confirmed. The results are expressed as mean ± SEM (n=3).

3.3 Effect of circSKA3 on the proliferation, migration, and invasion of HGSC cells

Proliferation, migration, and invasion are important aspects of cancer development and metastasis (Su *et al.*, 2019). That is why proliferation, wound healing, and invasion transwell assays were performed to study the function of circSKA3 in HGSC.

Based on the circRNA-seq data provided by our collogues and upregulation of circSKA3 in HGSC (Fig 3.1A), it was expected to see circSKA3 overexpression increases proliferation, migration, and invasion of OVCAR3 and CAOV3 cells. Conversely, it was expected that silencing circSKA3 in these cell lines would reverse the aforementioned effects.

For OVCAR3 cells overexpressed with circSKA3 (Fig. 3.3.1A), an increase in proliferation was observed at the time interval of 24 to 98 hours. An increase in proliferation also was seen for CAOV3 cells overexpressed with circSKA3 between time periods of 36 to 84 hours (Fig 3.3.1B). Silencing of circSKA3 with 20nM of siRNA concentration, in both OVCAR3 and CAOV3 cells, significantly reduced proliferation compared to NC transfected cells. Knockdown of circSKA3 in OVCAR3 decreased proliferation rate in a span of 24 – 54 hours (Fig 3.3.1C). Based on Figure 3.3.1D, in the periods of 24–59 hours lower proliferation for CAOV3 cells silent with circSKA3 was observed.

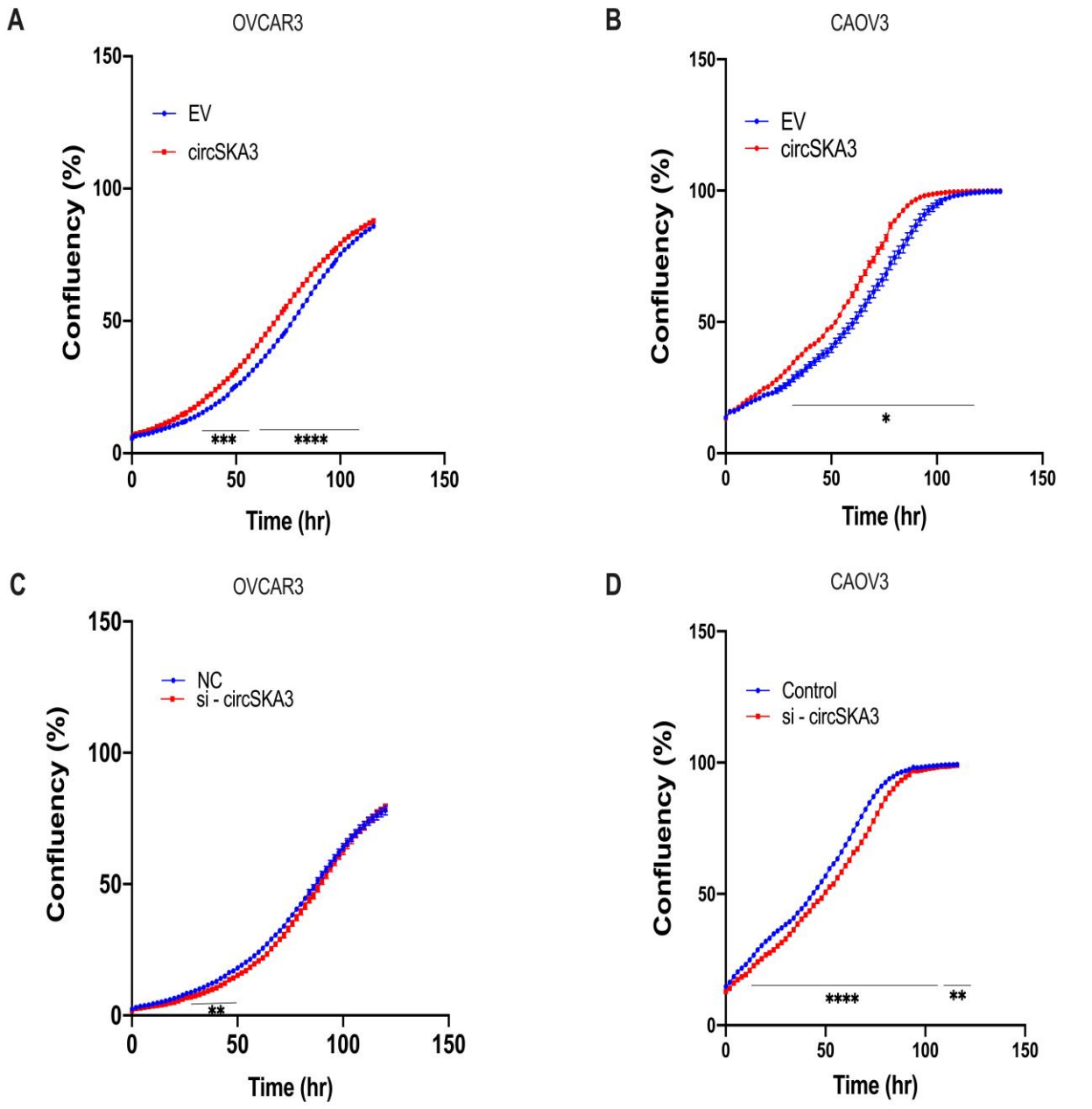

Figure 3.3.1: effect of circSKA3 on the proliferation of HGSC cells. OVCAR3 and CAOV3 cells were transiently transfected with each EV, circSKA3-expressing plasmids, NC and si-circSKA3. Overexpression of circSKA3 in OVCAR3 (A) and CAOV3 cells (B) led to higher proliferation. Whereas silencing of circSKA3 caused lower proliferation in OVACR3 (C) and CAOV3 (D) cells. Cell confluency was monitored using IncuCyte and the results are expressed as mean ± SEM in line graphs (n=20). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ represent statistical difference.

The same pattern was observed for migration as the overexpression of circSKA3 led to higher migration, while knocking down circSKA3 caused lower migration in HGSC cells. To start, overexpressing circSKA3 in OVCAR3 cells had a slight increase in migration rate compared to EV transfected cells after 56 hours (Fig. 3.3.2A). For CAOV3 cells overexpressed with circSKA3, a dramatic increase between time periods of 6 to 34 hours was observed (Fig 3.3.2B). In the periods of 10–48 hours, knockdown of circSKA3 in OVCAR3 cells reduced the migration rate (Fig 3.3.2 C). Based on figure 3.3.2D, after 6 hours lower migration rate for CAOV3 cells silent with circSKA3 was seen compared to the NC group.

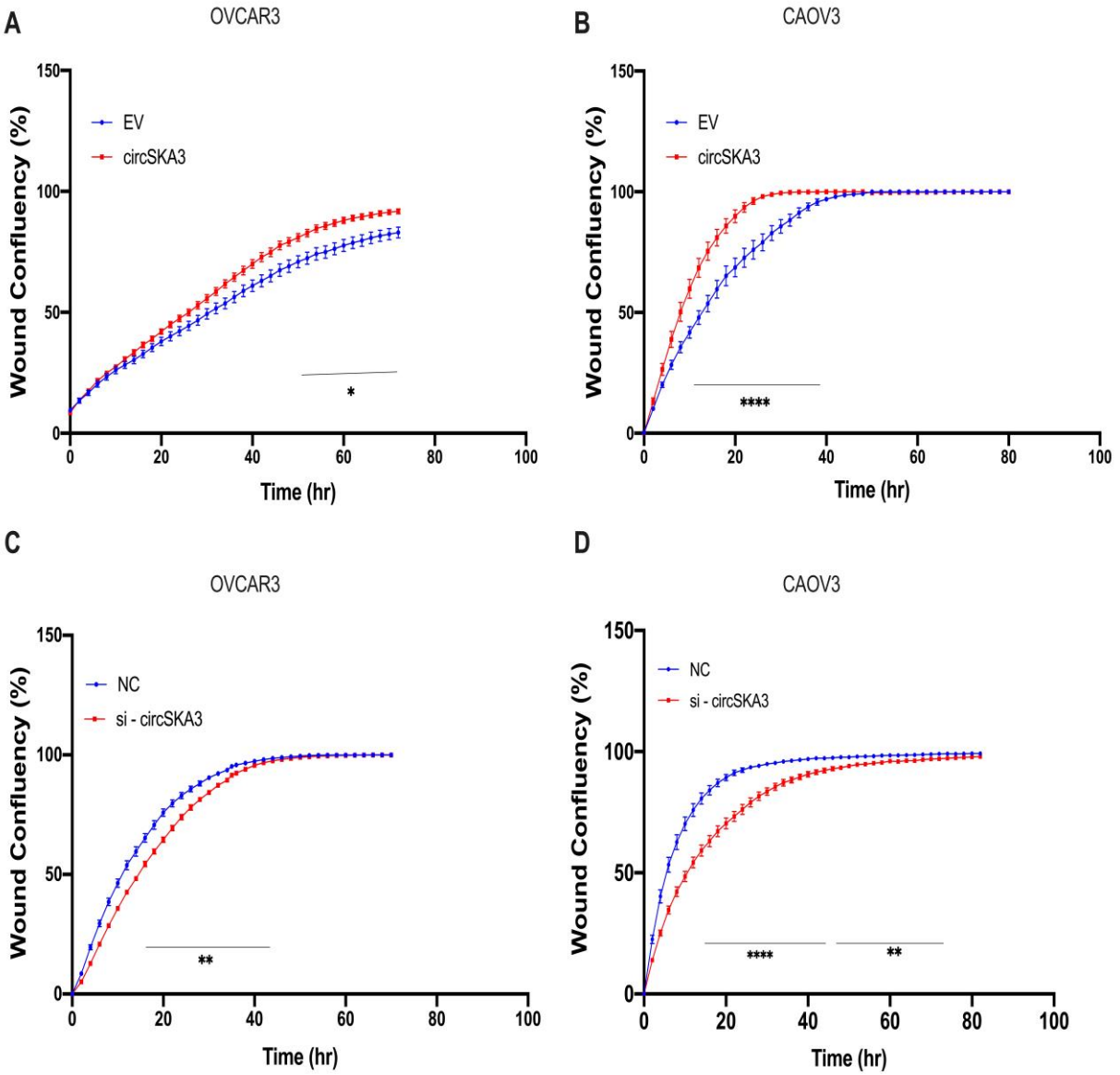

Figure 3.3.2: effect of circSKA3 on the migration of HGSC cells. OVCAR3 and CAOV3 cells were transiently transfected with each EV, circSKA3-expressing plasmids, NC and si-circSKA3. Overexpression of circSKA3 in OVCAR3 (A) and CAOV3 cells (B) enhanced migration. While silencing of circSKA3 led to lower migration in OVACR3 (C) and CAOV3 (D) cells. Wound confluency was monitored using IncuCyte and the results are expressed as mean ± SEM in line graphs (n=20). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ represent statistical difference.

Lastly, the invasion assays showed higher invasion in OVCAR3 (Fig 3.3.3 A) and CAOV3 (Fig 3.3.3 B) cells overexpressed with circSKA3 as compared to the EV transfected cells. In contrast to overexpression, fewer invaded cells were observed when circSKA3 was silenced in the OVCAR3 (Fig 3.3.3 C) and CAOV3 (3.3.3 D) cells compared to the NC group.

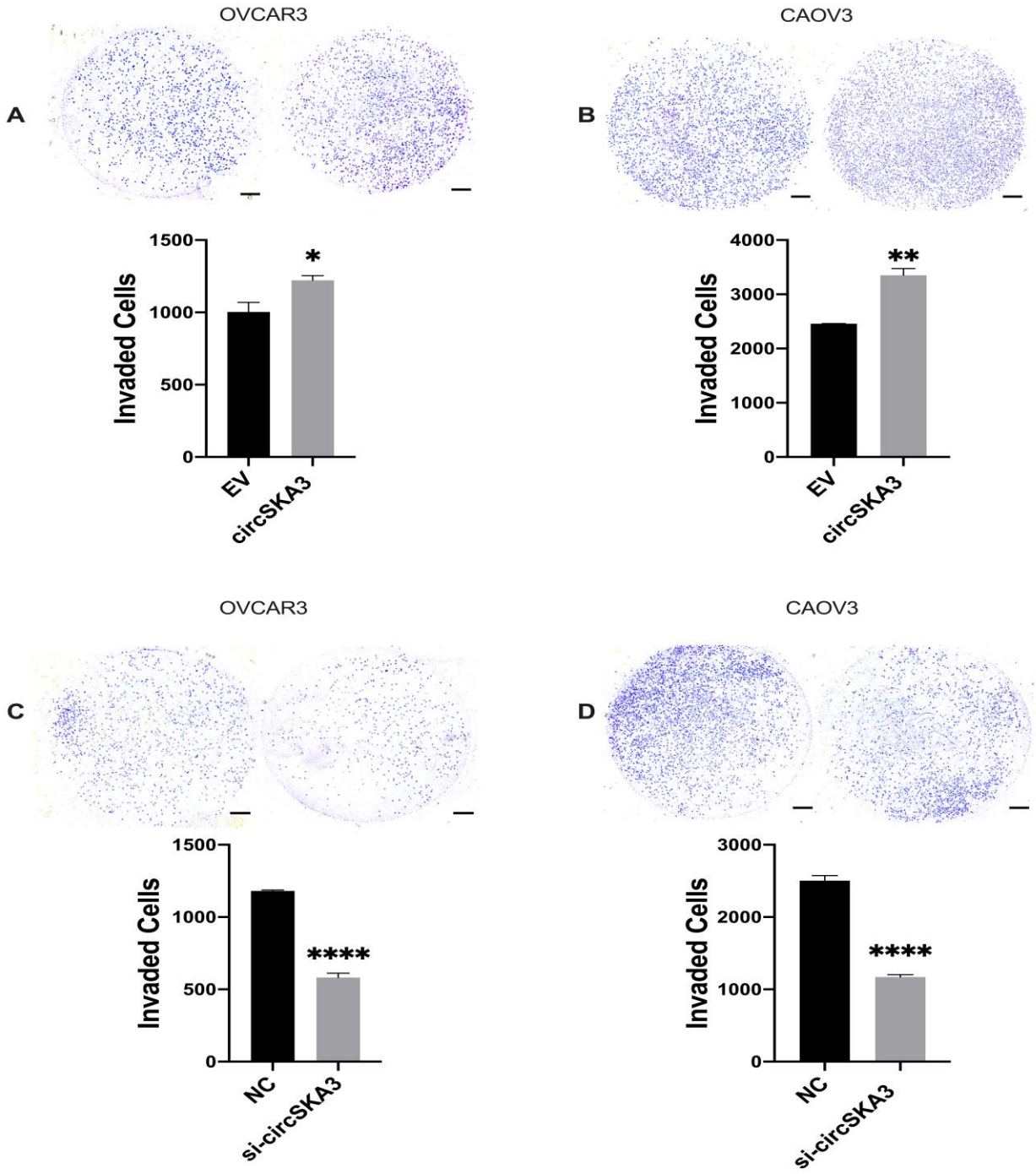

Figure 3.3.3: Effect of circSKA3 on the invasion of HGSC cells. Matrigel-coated transwell-invasion assays of transiently transfected OVCAR3 and CAOV3 cells with EV, circSKA3-expressing plasmids, NC and si-circSKA3. Overexpression of circSKA3 in OVCAR3 (A) and CAOV3 cells (B) enhanced invasion abilities. Silencing of circSKA3 led to fewer invaded cells in OVACR3 (C) and CAOV3 (D) cells. The results are expressed as mean ± SEM (n=3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ represent statistical difference.

3.4 Investigating the role of circSKA3 in the regulation of EMT markers

Several EMT marker expressions were examined due to the importance of EMT in driving cancer progression (Fang *et al.,* 2017). OVCAR3 and CAOV3 cells transiently transfected with plasmids (EV and circSKA3-expressing), or its siRNA were subjected to qPCR and western blot to investigate if circSKA3 regulates EMT markers at both mRNA and protein levels. Epithelial markers such as E-cadherin and mesenchymal marks such as N-cadherin, Snail, Slug, and Vimentin were examined.

Based on qRT-PCR data, the overexpression of circSKA3 in OVCAR3 resulted in upregulation of Snail and Slug expressions (Fig 3.4A). However only Snail expression was upregulated in CAOV3 overexpressed circSKA3 cells compared to EV transfected cells (Fig 3.4B). With regards to silencing circSKA3, at the mRNA level, none of the EMT markers showed any change in si–circSKA3 transfected OVCAR3 cells compared to NC transfected cells (Fig 3.4C). However, downregulation of Slug, Snail, and Vimentin expressions was observed in CAOV3 silenced with circSKA3 cells (Fig 3.4D).

Furthermore, a western blot was performed to confirm the expression of the EMT markers that showed changes at the mRNA level when overexpressed or silenced with circSKA3. At the protein level, upregulation of Snail and Slug expressions in OVCAR3 cells overexpressed with circSKA3 was observed compared to the EV transfected cells (Fig 3.4E). The protein levels of Snail, Slug, and Vimentin were reduced when CAOV3 cells were silenced with circSKA3 (Fig 3.4F).

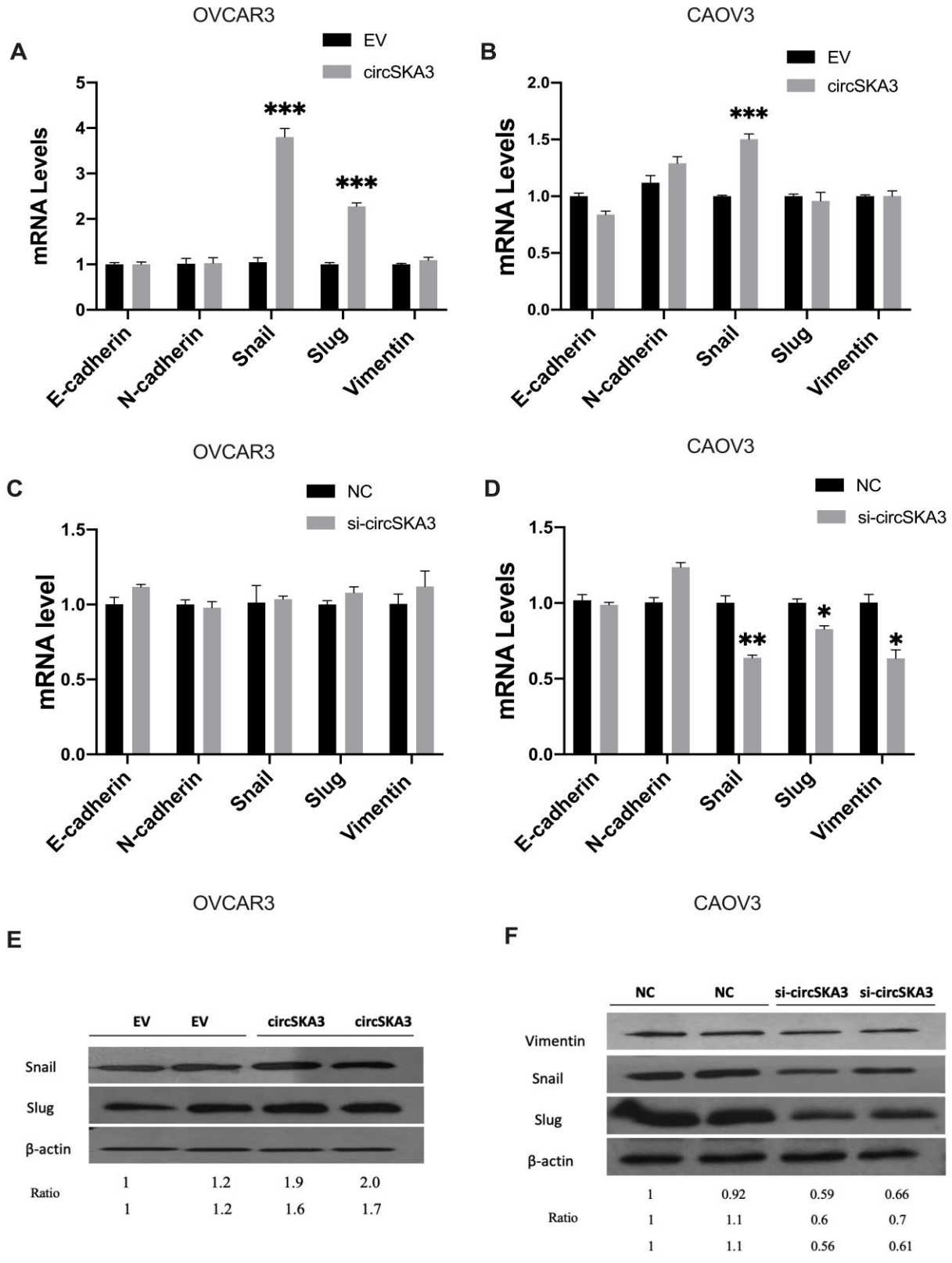

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Figure 3.4: Effect of circSKA3 in the regulation of EMT markers. RT-qPCR analysis of EMT markers mRNA level in transiently transfected OVCAR3 (A) and CAOV3 (B) cell lines overexpressed with circSKA3 and EV as well as OVCAR3 (C) and CAOV3 (D) cells transfected with si-circSKA3, and NC 48 hours post transfection. Western blot was used to assess the effect of circSKA3 on the protein levels of EMT markers 48 hours post transfection in OVCAR3 cells transfected with circSKA3-expressing and EV plasmids (E) and in CAOV3 cells transfected with si-circSKA3 and NC (F). Densitometry analysis of the circSKA3 protein bands normalized to that of β-actin. The results of RT-qPCR are expressed as mean ± SEM (n=3).

3.5 miR-383-5p as a potential target of circSKA3

The most common role reported for circRNAs, especially in cancer is to function as miRNAs sponges (Zhao *et al*., 2019). To determine if this applies to circSKA3 in EOC, using the CircInteractome database, 20 potential miRNAs target candidates were identified. Ten of the candidates with a context + score percentile above 90 were selected (Table 3.1). Among them, miR-383-5p and miR-382-3p were chosen because they have been reported to be downregulated in EOC and exert tumor-suppressing effects (Jiang *et al.*, 2019; Liu *et al.*, 2018), which are opposite to circSKA3. The expression levels of miR-383-5p and miR-382-3p were measured in the OVCAR3 and CAOV3 cells silenced with circSKA3 by RT-qPCR. Silencing circSKA3 in OVCAR3 (Fig 3.5A) and CAOV3 (Fig 3.5B) cells increased the expression of miR-383-5p; however, the expression of miR-382-3p was not changed. Therefore, we focused on Using EOC tissue samples from Ottawa Ovarian Cancer Tissue Bank (Supplementary Table S6.1), downregulation of miR-383-5p was observed in the HGSC compared to tumors of LMP (Fig 3.5D).

Table 3.1: Potential miRNAs target candidates and their target sites

Name	Target sites circSKA3 (Top) – miRNAs (Bottom)	Context + Score Percentile
Hsa-miR-579	AUAUACUAUGUGUUUAAAUGAAG |||||| UUAGCGCCAAAUAUGGUUUACUU	93
Hsa-miR-576-5p	GUGUGUAACUCCUAAAUUAGAAC ||||||| UUUCUGCACCUCUUUAAUCUUA	97
Hsa-miR-569	ACACGAGCAAGAAGCCAUUAACU ||||||| UGAAAGGUCCUAAGUAAUUGA	93
Hsa-miR-520h	ACUCCUAAAUUAGAACACUUUGG ||||| ||||||| UGAGAUUUCCCUUCGUGAAACA	99
Hsa-miR-382	GUCUCCACGUAGUCCACAACUUU ||||| ||||||| GCUUAGGUGGUGCU--UGUUGAAG	91
Hsa-miR-330-5p	AGCCAUUAACUCUGACCCAGAGU ||||||| CGGAUUCUGUGUCCGGGUCUCU	96
Hsa-miR-383	GUGAAAGAUGAUCUG--UCUGAUCC ||| ||||||| UCGGUGUUAGUGGAAGACUAGA	92
Hsa-miR-888	CUAAAAAUGGAUGAUUUUGAGUG ||||||| ACUGACUGUCGAAAAACUCAU	97
Hsa-miR-520g	ACUCCUAAAUUAGAACACUUUGG ||||| ||||||| UGUGAGAUUUCCCUUCGUGAAACA	99
Hsa-miR-1324	GAUGUGAAAGAUGAUCUGUCUGA ||||||| CUUUCACGUAUCUUAAGACAGACC	99

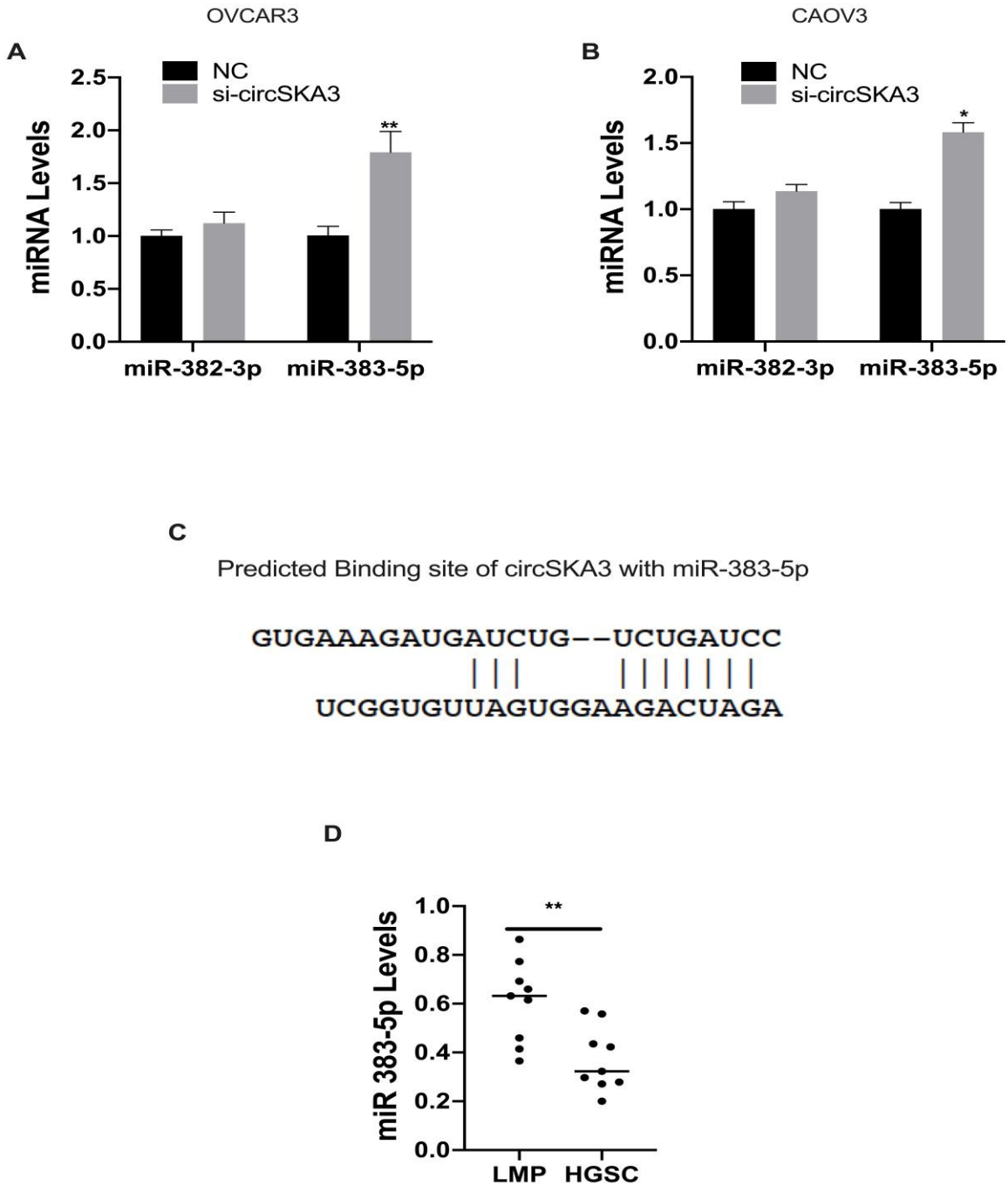


Figure 3.5: circSKA3 may target miR-383-5p. qRT-PCR analysis of the miR-383-5p and miR-382-3p expressions in OVCAR3 (A) and CAOV3 (B) cells transfected with NC and si-circSKA3 (n=3). A predicted binding site of circSKA3 with miR-383-5p is displayed (C). miR-383-5p levels were lower in high-grade serous ovarian tumors (HGSC, n = 10) than low malignancy potential tumors (LMP, n = 11) tested by qRT-PCR (D). The results are expressed as mean ± SEM. *, $P < 0.05$ and **, $P < 0.01$.

3.6 Overexpressing miR-383-5p attenuates the effect of circSKA3 on cell invasion and EMT marker genes expressions

To determine if circSKA3 exerts tumor promoting effects by inhibiting miR-383-5p, several experiments were performed to test if overexpression of miR-383-5p could reverse the effects of circSKA3.

First, cell invasion assays were carried out. OVCAR3 cells were transiently co-transfected with circSKA3-expressing plasmid or EV with miR-383-5p mimics or NC, and cells invaded through the Matrigel-coated insert were measured. As shown in Figure 3.6A, cells transfected with miR-383-5p mimics showed fewer invaded cells while cells overexpressed with circSKA3 had more invaded cells compared to the control group. We observed that introducing miR-383-5p mimics to the cells overexpressed with circSKA3 resulted in a significant decreased in the number of invaded cells when compared to cells overexpressing circSKA3 and the NC.

Next, the expression of Snail and Slug was measured by qPCR in circSKA3 and miR-383-5p-cotransfected cells and their controls. Overexpression of miR-383-5p significantly decreased Snail mRNA levels when compared with cells transfected with NC. Snail mRNA levels were higher in circSKA3-overexpressing cells (Fig 3.6B). However, the effect of circSKA3 in Snail expression was reversed when cells were co-transfected with miR-383-5p (Fig. 3.6B).

As for Slug expression levels, overexpression of miR-383-5p in OVCAR3 cells did not significantly alter Slug mRNA expression in cells transfected with EV. On the other hand, circSKA3 significantly induced Snail mRNA expression in cells transfected with NC, but not in cells transfected with miR-383-5p (Fig 3.6C), indicating that miR-383-5p overexpression reduced the effect of circSKA3 on Snail expression.

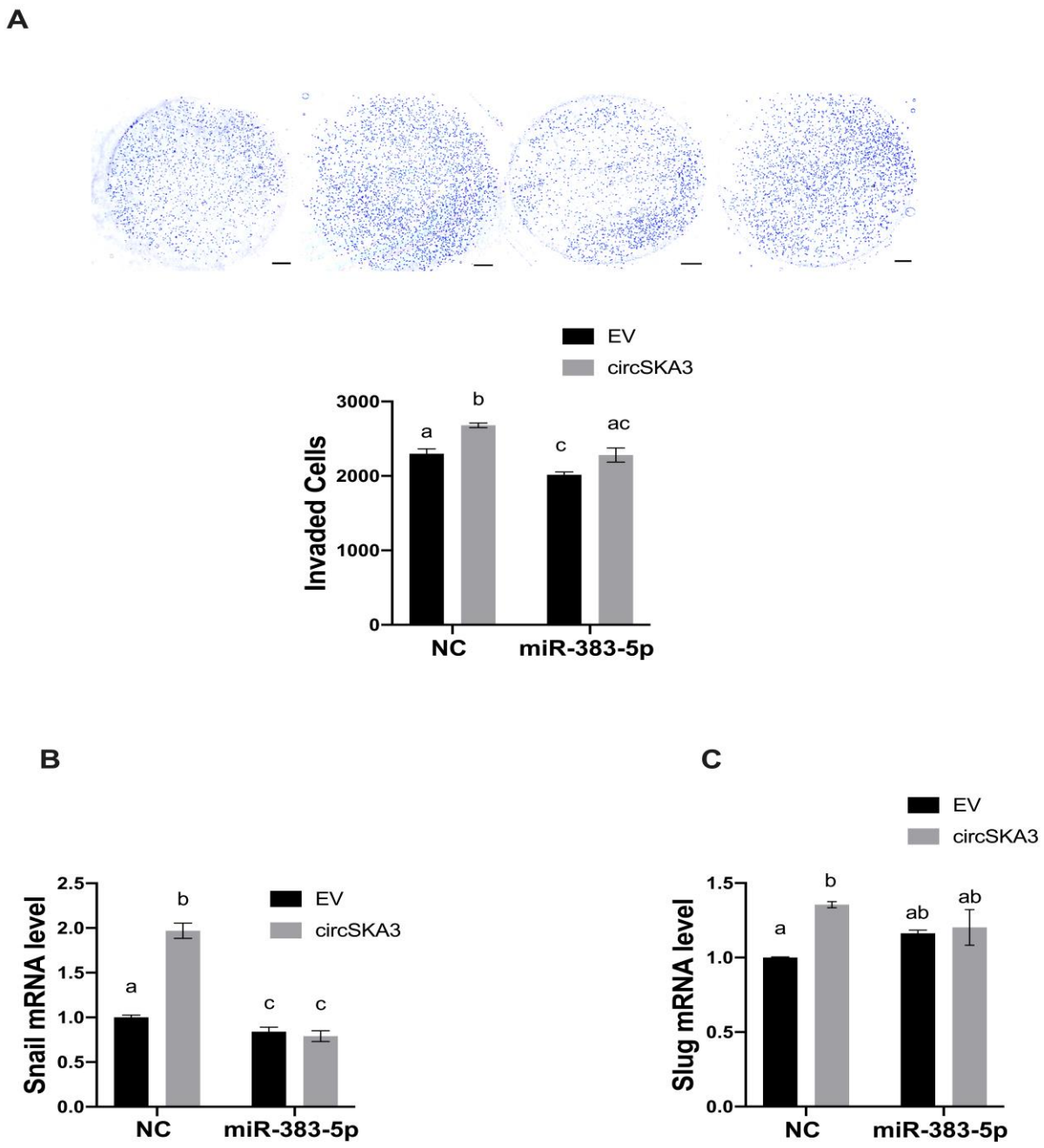


Figure 3.6: miR-383-5p reverses the promoting effect of circSKA3 on the invasion of OVCAR3 cells. Transwell invasion assay was performed for OVCAR3 cells co-transfected with circSKA3-expressing or EV plasmids with miR-383-5p mimics or NC (A). The expression of Snail and Slug were also measured in OVCAR3 cells co-transfected with circSKA3-expressing or EV plasmids with miR-383-5p mimics or NC by qRT-PCR (B & C). The results are expressed as mean ± SEM (n=3) and scale bars represent 1cm. Different letters denote statistically significant.

3.7 circSKA3 upregulates miR-383-5p target genes

To further confirm the regulation of miR-383-5p by circSKA3, I measured the mRNA level of several reported miR-383-5p target genes, including lactate dehydrogenase-A (LDHA), Stratifin (SFN), and c-Myc (Han *et al.,* 2017; Hu *et al.,* 2019; Tian *et al.,* 2013) in OVCAR3 and CAOV3 cells transfected with si-circSKA3 and NC by qRT-PCR. These target genes were chosen because they have been reported to be highly overexpressed in the EOC and have tumor-promoting effects (Han *et al.,* 2017; Hu *et al.*, 2019; Reyes-González *et al.,* 2015). As shown in Fig. 3.7, knockdown of circSKA3 reduced the mRNA expression levels of LDHA and SFN in both OVCAR3 (Fig. 3.7A-B) and CAOV3 (Fig. 3.7D-E) cells. but did not have any effect on the expression of c-Myc in either of the cells (Fig. 3.7C&F).

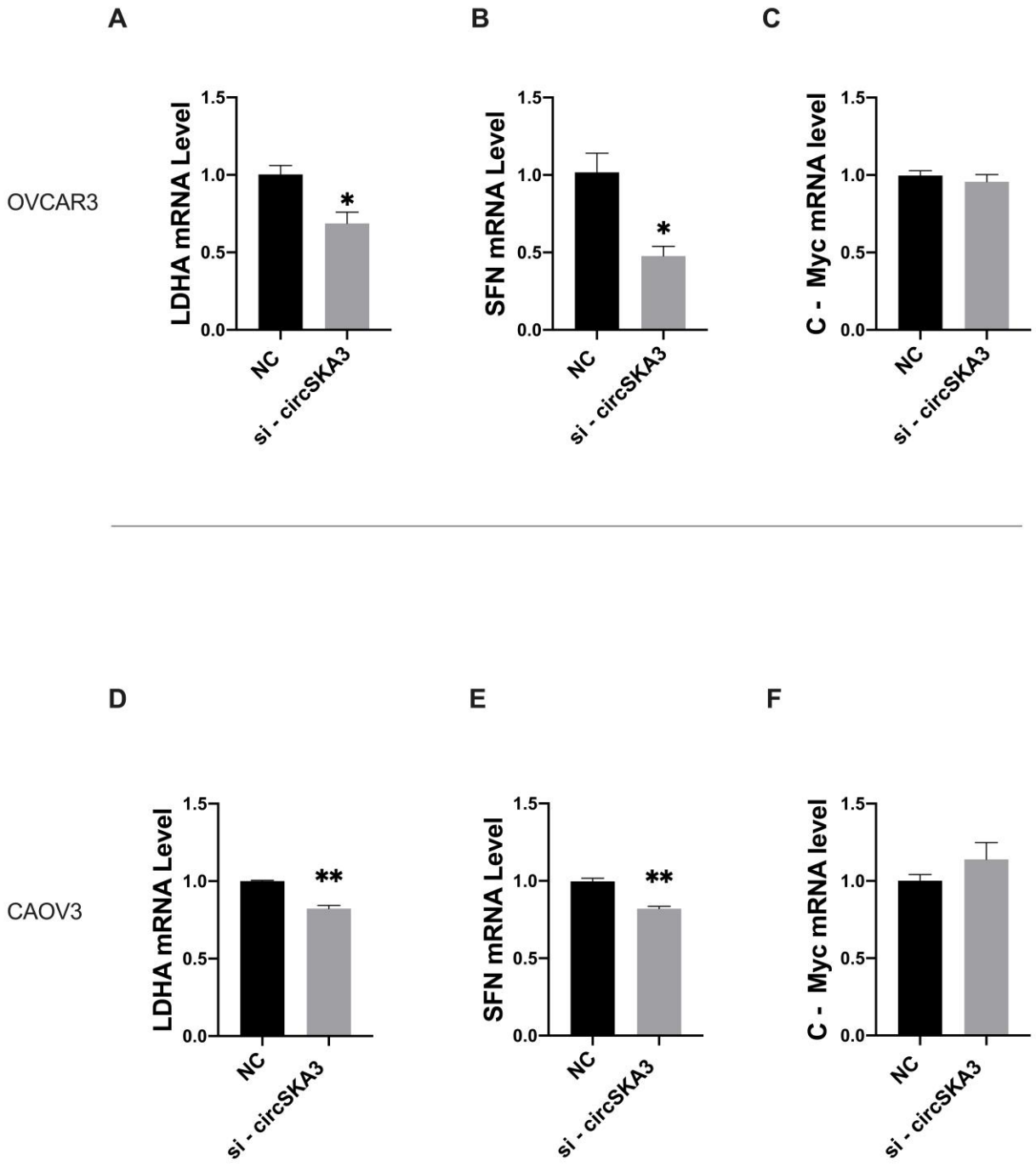


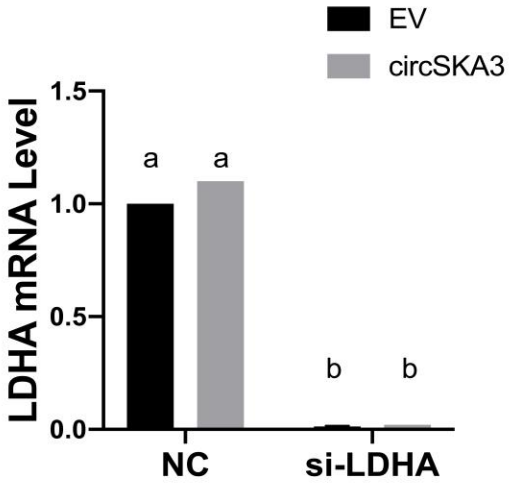
Figure 3.7: The impact of circSKA3 knockdown on the expression level of miR-383-5p mRNA targets. The expression levels of LDHA, SFN, and c-Myc in OVCAR3 (A-C) and CAOV3 (D-F) cells silenced with circSKA3. The results are expressed as mean ± SEM (n=3). *, $P < 0.05$ and **, $P < 0.01$ represent statistical difference.

3.8 LDHA may partially mediate the promoting effect of circSKA3 on cell invasion

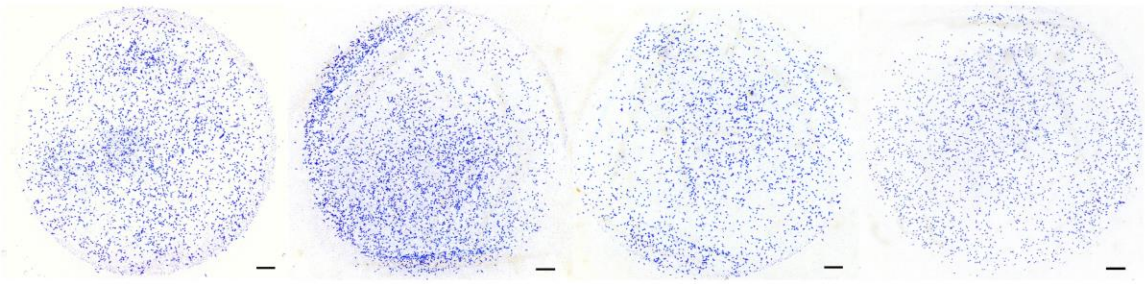
LDHA is one of the crucial regulators for tumor progression and invasion in many types of cancer, including EOC (Han *et al.*, 2017). Therefore, further experiments were done to determine if LDHA is a mediator of circSKA3. OVCAR3 cells were transiently co-transfected with circSKA3-expressing plasmid or its EV control, along with si–LDHA or NC. Following transfection, LDHA mRNA levels were measured to confirm the effectiveness of the LDHA siRNA. Indeed, transfection of si-LDHA significantly and strongly downregulated LDHA mRNA levels (Fig 3.8A).

Based on figure 3.8B, overexpression of circSKA3 in OVCAR3 cells showed high invasive ability while cells transfected with si-LDHA showed lower invasive ability compared to the control group. By introducing si-LDHA to the overexpressed circSKA3 OVACR3 cells, a lower invasive ability was observed compared to the overexpressed circSKA3 OVCAR3 cells.

A



B



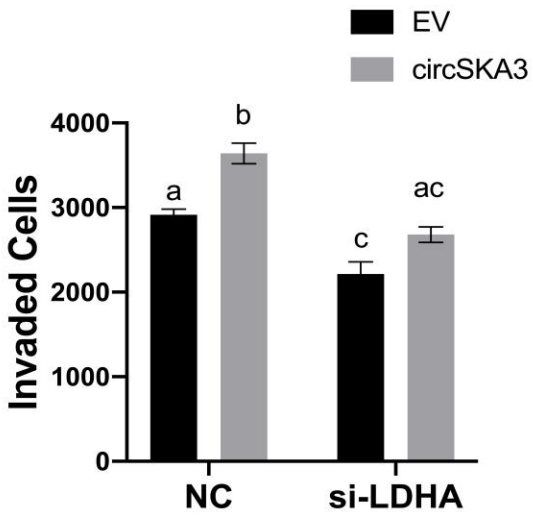


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Figure 3.8: Silencing LDHA blocks the promoting effect of circSKA3 on the invasion of OVCAR3 cells. The knockdown of LDHA was confirmed in OVCAR3 cells co-transfected with circSKA3-expressing or EV plasmids with si-LDHA or NC by qRT-PCR (A). Transwell invasion assay was performed for OVCAR3 cells co-transfected with circSKA3-expressing or EV plasmids with si–LDHA or NC (B). The results are expressed as mean ± SEM (n=3) and scale bars represent 1cm. Different letters denote statistical significance.

CHAPTER 4

DISCUSSION

4.1 circSKA exerts tumor-promoting effects in HGSC

Several studies have reported the tumor-promoting effect of circSKA3 in different cancers. However, the role and mechanism of circSKA3 in EOC have not previously been reported. In this study, we demonstrated that circSKA3 has tumor-promoting effects in HGSC, the most common and deadly subtype of EOC. We showed that circSKA3 was upregulated in HGSC tumor samples when compared with LMP tumors. This finding is consistent with circRNA-seq data provided by our collaborators that circSKA3 is significantly overexpressed in HGSC. These results suggest that circSKA3 is related to HGSC aggressive subtype.

Using several functional assays and two cell lines derived from HGSC tumors, we demonstrated that circSKA3 overexpression promotes proliferation, migration, and invasion in HGSC cells, while silencing circSKA3 displays an opposing effect in these tumor cell behaviors. Although there are no reports of the function of circSKA3 in EOC, our results are in agreement with previous studies, in which circSKA3 exerts its oncogenic effect by upregulating proliferation, migration, and invasion in other cancers. For example, in breast cancer, it was observed that knockdown of circSKA3 inhibited proliferation, migration, and invasion of cancer cells, while overexpression of circSKA3 reversed the aforementioned effects (Du *et al.*, 2020). Similarly, in gastric and colorectal cancers, knockdown of circSKA3 inhibited cell malignant behaviors such as proliferation and invasion (Lu *et al*, 2019; Xie & Pan, 2021). Our findings support a tumor-promoting role of circSKA3 in HGSC. Future studies using in vivo models are required to confirm the role of circSKA3 in HGSC development.

Gaining EMT features correlates with cancer progression and metastasis and contributes to different hallmarks of cancer including proliferation, migration, and invasion (Son and Mon *et al.*, 2015; Kariya *et al.*, 2021). EMT is a process in which cells gain mesenchymal features by

suppressing their epithelial features to acquire mobility and migration ability (Ramos *et al.*, 2017). EMT is regulated by multiple transcription factors. Suppression of epithelial markers such as E-cadherin and upregulation of mesenchymal markers such as Snail, Slug, and Vimentin enables EMT to occur. Snail and Slug are transcription factors that have crucial roles in the induction of EMT because they are the main effectors of many EMT inducers and repressors (Wang *et al*., 2019). Importantly, they are involved in the initiation of EMT (Medici *et al.*, 2008). Considered to be one of the transcriptional regulators of E-cadherin, they can down-regulate E-cadherin expression and enhance the expression of Vimentin to induce EMT (Wang *et al.*, 2019). In their turn, Vimentin provides structural and functional support for cancer cells while loss of E-cadherin leads to dissociation of epithelial cells from their origin (Usman *et al*., 2021: Son and Moon *et al.*, 2010). By losing E-cadherin, cancer cells gain N-cadherin expression to enhance their motility, invasion, and metastasis (Chung *et al*., 2013). Moreover, Snail and Slug collaboratively induce EMT to promote the invasion and migration of cancer cells (Zheng *et al.*, 2015). In this study, we found that Snail and Slug expressions were upregulated in OVCAR3 cells overexpressing circSKA3 while upregulation of only Snail expression was observed in CAOV3 cells overexpressing circSKA3. Knockdown of circSKA3 downregulated EMT markers such as Vimentin, Snail, and Slug in circSKA3-silencing CAOV3 cells. However, none of the EMT markers expressions changed in OVCAR3 cells silent with circSKA3. The differences in EMT markers in both OVCAR3 and CAOV3 cells might be due to the sensitivity of cells to circSKA3 overexpression or knockdown. OVCAR3 cells had lower endogenous circSKA3 levels than CAOV3 cells, which is why they might be more sensitive to circSKA3 overexpression. In contrast, CAOV3 cells had the highest endogenous circSKA3 level, which might explain their higher

sensitivity to circSKA3 knockdown. These findings suggest that circSKA3 may promote the initiation of EMT by upregulating Snail and Slug to exert its tumor-promoting effect in HGSC.

4.2. circSKA3 inhibits miR-383-5p

One of the best-known functions of circRNAs is that they behave as miRNAs sponges (Zhang *et al.,* 2019). This means that circRNAs can inhibit the function of miRNAs by binding to them to block their functions, thereby indirectly regulating the expression of their target genes. Depending on the cancer type, circSKA3 has been shown to sponge a variety of miRNAs to promote cancer progression. For example, circSKA3 sponges miR-326-5p in gastric cancer while it sponges miR-1 in glioblastoma (Mo *et al.,* 2020; Zhou *et al.,* 2021). Among potential miRNA targets for circSKA3, the expression levels of tumor-suppressive miR-383-5p and miR-382-3p in EOC were examined (Jiang *et al.,* 2019; Liu *et al.,* 2018). We found that only miR-383-5p levels were upregulated after silencing circSKA3 in HGSC cells. Interestingly, circSKA3 promotes medulloblastoma progression by sponging miR-383-5p (Wang *et al.,* 2020). CircSKA3 likely uses a similar mechanism in EOC as well. However, further studies are needed to confirm the sponging of miR-383-5p by circSKA3 in EOC. Studies such as pull-down assay will be performed to see whether miR-383-5p will be pulled down by circSKA3 in OVCAR3 or CAOV3 cells.

Previous reports showed that miR-383-5p suppresses invasion and proliferation but induces apoptosis in several types of cancers, including EOC (Jiang *et al.,* 2019). Locating at chromosome 8, miR-383-5p has shown to target different genes in the same cancer to exerts its tumor suppressing effect. For example, in the EOC, miR-383-5p suppressed the proliferation and enhanced the chemosensitivity of cancer cells by targeting TRIM27 (Jiang *et al.,* 2019). Han *et al.* (2017) study showed miR-383-5p also targets LDHA to reduce EOC cell proliferation, invasion,

and aerobic glycolysis. Consistent with these findings, we also observed the downregulation of the miR-383-5p level in HGSC compared to the LMP and the inhibition of cell invasion when miR-383-3p was overexpressed. Next, the rescue invasion assay gave us a better understanding of the regulatory relationship between circSKA3 and miR-383-5p. We demonstrated that overexpression of miR-383-5p reduced the tumor-promoting effect of circSKA3 on invasion and EMT markers such as Snail. This finding suggests that downregulation of miR-383-5p is responsible, at least in part, for the tumor-promoting effect of circSKA3 in HGSC. In other words, circSKA3 may promote EOC progression by suppressing miR-383-5p. Again, our results are in harmony with Wang *et al.* (2020) that the inhibitory effect of si-circSKA3 on the invasion was partially reversed by miR-383-5p inhibitor in medulloblastoma. Examination of Snail and Slug mRNA levels revealed that miR-383-5p reversed circSKA3-induced Snail and Slug expression. However, while miR-383-5p significantly reduced Snail mRNA levels, the expression of Slug was not affected by miR-383-5p transfection. Based on two databases' predictions (TargetScan and miRDB), Slug and Snail are not predicted targets of miR-383-5p. They were not among the EMT markers that were regulated by miR-383-5p in cancers such as head and neck squamous cell carcinoma and breast cancer at the mRNA or protein levels (Wang *et al.*, 2021; Azarbarzin *et al.*, 2021). In spite of that, Snail expression was suppressed by downregulation of the LDHA in renal cell carcinoma (Zhao *et al.*, 2017). It is possible that miR-383-5p affects the expression of Snail by regulating LDHA.

4.3. circSKA3 upregulates LDHA

Given that circSKA3 may regulate miR-383-5p expression, we tested if two of the verified miR-383-5p targets, LDHA and SFN, were also affected by circSKA3 and found that both were

downregulated in OVCAR3 and CAOV3 cells transfected with circSKA3 siRNA. In EOC, it has been reported LDHA is a target of miR-383-5p (Han *et al.,* 2017) and therefore, we further determined the role of LDHA in mediating the tumor-promoting effects of circSKA3. SFN is involved in the cell cycle checkpoint and is upregulated in the EOC (Hu *et al.*, 2019). Although the function and mechanism of SFN in EOC are still unclear, knockdown of SFN reduced proliferation, invasion, and migration of cervical cancer cells. Interestingly, SFN is known to be targeted by miR-383-5p in cervical cancer (Hu *et al.*, 2019). It is possible that circSKA3 may promote cancer progression by sponging miR-383-5p to upregulate SFN. This will be investigated in future studies.

LDHA is a glycolytic enzyme that catalyzes the conversion of pyruvate into lactate to provide energy for the cells without the need for oxygen (Feng *et al*., 2018). Malignant tumor cells switch to aerobic glycolysis for more energy by upregulating LDHA in order to survive and increase their malignancy behaviors such as proliferation and invasion (Zhu *et al*., 2018). On the other hand, LDHA inhibition will lead to a lower energy supply in cancer cells which in return reduces invasion and metastasis (Zhu *et al*., 2018). Several studies highlighted the role of LDHA as a key tumor-promoting regulator in many cancers, such as EOC. Hou *et al*. (2021) reported that the knockdown of LDHA significantly attenuates tumor progression and metastasis by deregulation of intracellular acetyl-CoA. Reduction in intracellular acetyl-CoA in turn reduces the histone acetylation of EMT-related genes which leads to lower proliferation and invasion of cancer cells of papillary thyroid carcinoma (Hou *et al.*, 2021). In this study, we found that silencing LDHA counteracted the promoting effect of circSKA3 on the OVCAR3 cells invasion, suggesting that LDHA may also contribute to the tumor-promoting effect of circSKA3 in HGSC. Knockdown of LDHA likely reverses the promoting effect of circSKA3 by deregulating intracellular acetyl-

CoA. However, more studies are needed to understand the regulatory relationship between circSKA3 and LDHA. Although LDHA knockdown reversed the effect of circSKA3 on cell invasion, circSKA3 overexpression did not show any change in the mRNA level of LDHA in OVCAR3 cells. In this project, all experiments were carried out 48 hours after transient transfection. No change was observed in the LDHA level after overexpression of circSKA3 and this might be due to the time limitation of transient transfection. In other words, overexpression of circSKA3 might need more than 48 hours to regulate the expression of LDHA which is why in the future the expression of LDHA must be checked 72 and 96 hours after transient transfection.

Overall, in this thesis, we have demonstrated that circSKA3 can have a tumor-promoting effect in HGSC by inducing proliferation, migration, and invasion. Our findings also suggest that one of the mechanisms by which circSKA3 exerts these tumor-promoting effects is to sponge miR-383-5p and in turn, upregulates LDHA (Fig 4.1). However, more studies are required to confirm the role of circSKA3 in HGSC progression and to comprehensively examine the mechanisms underlying the tumor-promoting actions of circSKA3.

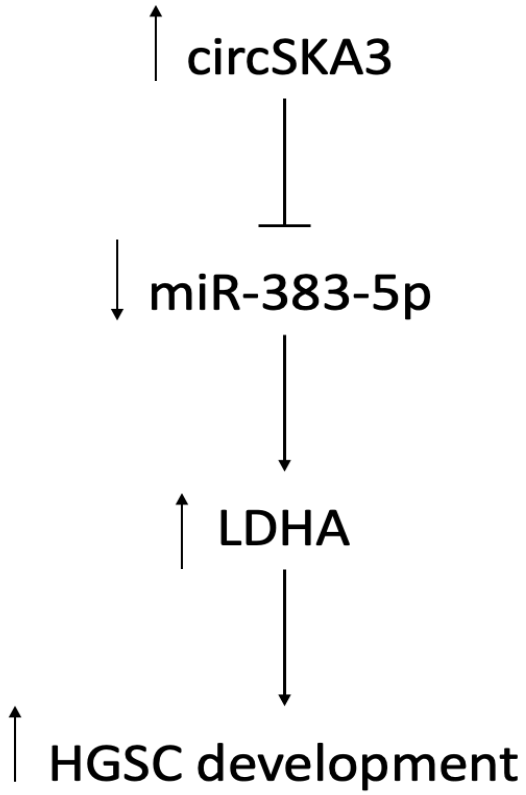

Figure 4.1: Proposed mechanism of circSKA3 in EOC. One possible way that circSKA3 promotes HGSC development is by sponging miR-383-5p which in turn upregulates LDHA.

CHAPTER 5

FUTURE DIRECTIONS AND CONCLUSION

5.1 Future Directions

More research is needed to fulfill the objectives of the study in the future. To begin with, loss and gain of function assays must be performed on other EOC cell lines such as SKOV3.ip1, a model of endometrioid carcinoma, to conclude that circSKA3 acts as a tumor-promoting effector in OC. To confirm that circSKA3 sponges miR-383-5p, a pull-down assay will be conducted to see whether the circSKA3 directly interacts with miR-383-5p. After confirmation of interaction, the miR-383-5p should also be overexpressed or silenced to see if it will affect the expression of the circSKA3. To see whether the knockdown of LDHA blocks the action of circSKA3 on the invasion by reducing intracellular acetyl-CoA, the level of intracellular acetyl-CoA will be measured in the cells co-transfected with circSKA3-expressing or EV plasmids, and si-LDHA or NC cells. In addition, EMT markers expressions also will be checked in the mentioned cells.

Generating stable cell lines using shRNAs that target the junction site of circRNA to knockdown circSKA3 in different OC cell lines is also an important step to take for experiments such as *in vivo* studies. Lastly, *in vivo* studies will also be conducted to examine the effect of circSKA3 on tumor progression using stably circSKA3 knockdown plasmids. Mouse models can be injected intraperitoneally with OVCAR3 or CAOV3 control or si-circSKA3 stable cells. Then, the size and weight of tumors will be measured.

5.2 Conclusion

This Study validated that circSKA3 is upregulated and has a tumor-promoting effect in the HGSC. Its overexpression increased proliferation, migration, and invasion of OVCAR3 and CAOV3 cells while silencing circSKA3 reduced proliferation, migration, and invasion of those cells. CircSKA3 was also involved in the regulation of some of the EMT markers at both mRNA and protein levels. For the knockdown of circSKA3 in CAOV3 cells, downregulation of Vimentin, Slug, and Snail expressions was observed. For the overexpression of circSKA3 in OVCAR3 cells, upregulation of Snail and Slug levels was recorded.

Regarding its mechanism of action, circSKA3 may act as a miRNA sponge for miR-383-5p to regulate LDHA expression. Knockdown of circSKA3 increases miR-383-5p levels and suppresses the mRNA levels of the miR-383-5p targets such as LDHA and SFN. Furthermore, miR-383-5p treatment attenuated the effect of circSKA3 on cell invasion and the EMT marker gene expressions such as Snail. LDHA is also involved in the tumor-promoting effect of circSKA3. Silencing of LDHA blocks the effect of circSKA3 on cell invasion.

In conclusion, our study showed that circSKA3 promotes HGSC development by suppressing miR- 383-5p to induce LDHA expression. Continuing this project is important, as studying circSKA3 will give a better understanding of the implication of SKA3 in EOC. Considering the results obtained from this project, circSKA3 may be used as a novel biomarker for screening EOC and may become a potential therapeutic target in the future.

BIBLIOGRAPHY

Abad, M. A., Zou, J., Medina-Pritchard, B., Nigg, E. A., Rappsilber, J., Santamaria , A., & Jeyaprakash , A. A. (2016). Ska3 Ensures Timely Mitotic Progression by Interacting Directly With Microtubules and Ska1 Microtubule Binding Domain. *Scientific reports, 6*(1), 1-9.

Acunzo, M., Romano, G., Wernicke, D., & Croce, C. M. (2015). MicroRNA and cancer--a brief overview. *Advances in biological regulation, 57*, 1-9.

Azarbarzin, S., Hosseinpour-Feizi, M., Khojasteh, S., Baradaran, B., & Safaralizadeh, R. (2021). MicroRNA -383-5p restrains the proliferation and migration of breast cancer cells and promotes apoptosis via inhibition of PD-L1. *Life Sciences, 267*.

Babaier, A., & Ghatage, P. (2020). Mucinous Cancer of the Ovary: Overview and Current Status. *Diagnostics (Basel, Switzerland), 10*(1), 52.

Bai, S., Wu, Y., Yan, Y., Shao, S., Zhang, J., Liu, J., . . . Ren, J. (2020). Construct a circRNA/miRNA/mRNA regulatory network to explore potential pathogenesis and therapy options of clear cell renal cell carcinoma. *Scientific Reports, 10*(1), 1-15.

Bonome, T., Lee, J.-Y., Park, D. C., Radonovich, M., Masison, C. P., Brady, J., . . . Birrer, M. J. (2005). Expression Profiling of Serous Low Malignant Potential, Low-Grade, and High-Grade Tumors of the Ovary. *Cancer Res, 65*(22), 10602–10612.

Chung, S., Yao, J., Suyama, K., Bajaj, S., Qian, X., Loudig, O., . . . Hazan, R. (2013). N-cadherin regulates mammary tumor cell migration through Akt3 suppression. *Oncogene, 32*, 422–430.

Das, A., Sinha, T., Shyamal , S., & Panda , A. C. (2021). Emerging Role of Circular RNA-Protein Interactions. *Non-coding RNA, 7*(3), 48.

Du, W. W., Li, X., Ma, J., Fang, L., Wu, N., Li, F., . . . Yang, B. B. (2021). Promotion of tumor progression by exosome transmission of circular RNA circSKA3. *Molecular therapy. Nucleic acids, 27*, 276-292.

Gadducci, A., & Cosio, S. (2020). Therapeutic Approach to Low-Grade Serous Ovarian Carcinoma: State of Art and Perspectives of Clinical Research. *Cancers, 12*(5), 1336.

Han, C., Seebacher, N. A., Hornicek, F. J., Kan, Q., & Duan, Z. (2017). Regulation of microRNAs function by circular RNAs in human cancer. *Oncotarget, 8*(38), 64622–64637.

He, A. T., Liu, J., Li , F., & Yang, B. B. (2021). Targeting circular RNAs as a therapeutic approach: current strategies and challenges. *Signal Transduction and Targeted Therapy, 6*(1), 1-14.

Hou, X., Shi, X., Zhang, W., Li , D., Hu , L., Yang, J., . . . Gao, M. (2021). LDHA induces EMT gene transcription and regulates autophagy to promote the metastasis and tumorigenesis of papillary thyroid carcinoma. *Cell death & disease, 12*(4).

Kariya, Y., Oyama, M., Suzuki , T., & Kariya, Y. (2021). αvβ3 Integrin induces partial EMT independent of TGF-β signaling. *Communications biology, 4*(1), 490.

Li, B., Cai, X., Wang, Y., Zhu, H., Zhang, P., Jiang, P., . . . Shao , L. (2021). Circ-SKA3 Enhances Doxorubicin Toxicity in AC16 Cells Through miR-1303/TLR4 Axis. *International heart journal, 62*(5), 1112-1123.

Lisio, M. A., Fu, L., Goyeneche, A., Gao, Z.-h., & Telleria, C. (2019). High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. *Int. J. Mol. Sci., 20*(4), 952.

Pierson, W. E., Peters, P. N., Chang, M. T., Quigley, D. A., Ashworth, A., & Chapman, J. S. (2020). An integrated molecular profile of endometrioid ovarian cancer. *Gynecologic Oncology, 157*(1), 55-61.

Reyes-González, J. M., Armaiz-Peña, G. N., Mangala , L. S., Valiyeva, F., Ivan, C., Pradeep, S., . . . Vivas-Mejía, P. E. (2015). Targeting c-MYC in Platinum-Resistant Ovarian Cancer. *Molecular cancer therapeutics, 14*(10), 2260-9.

Serrano-Gomez, S. J., Maziveyi, M., & Alahari , S. K. (2016). Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *Molecular cancer, 15*, 18.

Vo, J. N., Cieslik, M., Zhang, Y., Shukla, S., Xiao, L., Zhang, Y., . . . Chinnaiyan, A. M. (2019). The Landscape of Circular RNA in Cancer. *Cell, 176*(4), 869-881.

Williams, T. I., Toups, K. L., Saggese, D. A., Kalli, K. R., Cliby, W. A., & Muddiman, D. C. (2007). Epithelial Ovarian Cancer: Disease Etiology, Treatment, Detection, and Investigational Gene, Metabolite, and Protein Biomarkers. *Journal of proteome research, 6*(8), 2936-2962.

Yang-Hartwich, Y., Soteras, M., Sumi, N., Joo, W., Holmberg, J. C., Craveiro, V., . . . Mor , G. (2014). Ovulation and extra-ovarian origin of ovarian cancer. *Scientific reports, 4*, 6116.

Yi, Q., Xie, W., Sun, W., Sun, W., & Liao, Y. (2022). A Concise Review of MicroRNA-383: Exploring the Insights of Its Function in Tumorigenesis. *Journal of Cancer, 13*(1), 313-324.

Zhao, X., Cai, Y., & Xu, J. (2019). Circular RNAs: Biogenesis, Mechanism, and Function in Human Cancers. *International journal of molecular sciences, 20*(16), 3926.

Zhou, M., Li, H., Chen, K., Ding, W., Yang, C., & Wang, X. (2021). CircSKA3 Downregulates miR-1 Through Methylation in Glioblastoma to Promote Cancer Cell Proliferation. *Cancer management and research, 13*, 509–514.

Arnaiz, E., Sole, C., Manterola, L., Iparraguirre, L., Otaegui, D., & Lawrie, C. H. (2019). CircRNAs and cancer: Biomarkers and master regulators. *In Seminars in cancer biology, 58*, 90-99.

Barrett , S. P., & Salzman, J. (2016). Circular RNAs: analysis, expression and potential functions. *Development, 143*(11), 1838-47.

Cheng, W., Liu, J., Yoshida, H., Rosen, D., & Naora, H. (2005). Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. *Nature medicine, 11*(5), 531-7.

Colombo, N., Gorp, T. V., Parma, G., Amant , F., Gatta, G., Sessa, C., & Vergote, I. (2006). Ovarian cancer. *Critical reviews in oncology/hematology, 60*(2), 159-179.

Committee on the State of the Science in Ovarian Cancer Research, B. o. (2016). *Ovarian Cancers: Evolving Paradigms in Research and Care.* National Academies Press (US).

Desai, A., Xu, J., Aysola, K., Qin, Y., Okoli, C., Hariprasad, R., . . . Gr. (2014). Epithelial ovarian cancer: An overview. *World journal of translational medicine, 3*(1), 1-8.

Devouassoux-Shisheboran, M., & Genestie , C. (2015). Pathobiology of ovarian carcinomas. *Chinese journal of cancer, 34*(1), 50-55.

Dragomir, M., & Calin, G. A. (2018). Circular RNAs in Cancer – Lessons Learned From microRNAs. *Frontiers in oncology, 8*, 179.

Du, W. W., Yang, W., Li, X., Fang, L., Wu, N., Li, F., . . . Yang, B. B. (2020). The Circular RNA circSKA3 Binds Integrin β1 to Induce Invadopodium Formation Enhancing Breast Cancer Invasion. *Molecular therapy, 28*(5), 1287–1298.

Fang, D., Chen, H., Zhu, J., Wang, W., Teng, Y., Ding, H.-F., . . . Huang, S. (2017). Epithelial–mesenchymal transition of ovarian cancer cells is sustained by Rac1 through simultaneous activation of MEK1/2 and Src signaling pathways. *Oncogene, 36*(11), 1546–1558.

Feeney, L., Harley, I. J., McCluggage, W. G., Mullan, P. B., & Beirne , J. P. (2020). Liquid biopsy in ovarian cancer: Catching the silent killer before it strikes. *World J Clin Oncol , 11*(11), 868-889.

Feng, Y., Xiong, Y., Qiao, T., Li, X., Jia, L., & Han, Y. (2018). Lactate dehydrogenase A: A key player in carcinogenesis and potential target in cancer therapy. *Cancer medicine, 7*(12), 6124-6136.

Gao, W., Zhang, Y., Luo, H., Niu, M., Zheng, X., Hu, W., . . . Z. (2020). Targeting SKA3 suppresses the proliferation and chemoresistance of laryngeal squamous cell carcinoma via impairing PLK1-AKT axis-mediated glycolysis. *Cell death & disease, 11*(10), 919.

Geng, X., Jia, Y., Zhang, Y., Shi , L., Li, Q., Zang, A., & Wang, H. (2020). Circular RNA: biogenesis, degradation, functions and potential roles in mediating resistance to anticarcinogens. *Epigenomics, 12*(3), 267-283.

Han, R. L., Wang, F. P., Zhang , P. A., Zhou, X. Y., & Li, Y. (2017). miR-383 inhibits ovarian cancer cell proliferation, invasion and aerobic glycolysis by targeting LDHA. *64*(2), 244-252.

Hu, Y., Ma, Y., Liu, J., Cai, Y., Zhang, M., & Fang , X. (2019). LINC01128 expedites cervical cancer progression by regulating miR-383-5p/SFN axis. *BMC CANCER, 19*(1), 1157.

Hu, Y., Zeng, Q., Li, C., & Xie, Y. (2019). Expression profile and prognostic value of SFN in human ovarian cancer. *Bioscience reports, 39*(5), BSR20190100.

Huang, A., Zheng, H., Wu, Z., Chen, M., & Huang , Y. (2020). Circular RNA-protein interactions: functions, mechanisms, and identification. *Theranostics, 10*(8), 3503.

Jiang, J., Xie, C., Liu, Y., Shi, Q., & Chen, Y. (2019). Up-regulation of miR-383-5p suppresses proliferation and enhances chemosensitivity in ovarian cancer cells by targeting TRIM27. *Biomedicine & Pharmacotherapy, 109*, 595-601.

Jiang, M., Fang, S., Zhao, X., Zhou , C., & Gong, Z. (2021). Epithelial-mesenchymal transition-related circular RNAs in lung carcinoma. *Cancer Biology & Medicine, 18*(2), 411–420.

Jin, L., Chun, J., Pan, C., Alesi, G., Li, D., Magliocca, K., . . . Kang, S. (2017). Phosphorylation-mediated activation of LDHA promotes cancer cell invasion and tumour metastasis. *Oncogene, 36*(27), 3797-3806.

Kim, J., Coffey, D. M., Creighton, C. J., Yu, Z., Hawkins, S. M., & Matzuk, M. M. (2012). High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proceedings of the National Academy of Sciences of the United States of America,, 109*(10), 3921–3926.

Kishorbhai, V., & Malaviya, M. (2021). OVERVIEW OF AN OVARIAN CANCER AND ITS TREATMENT ASPECTS. *International Journal of Pharmaceutical and Biological Science Archive, 9*(2).

Kristensen, L. S., Andersen, M. S., Stagsted, L., Ebbesen, K. K., Hansen, T. B., & Kjems , J. (2019). The biogenesis, biology and characterization of circular RNAs. *Nature Reviews Genetics, 20*(11), 675-691.

Le, A., Cooper, C. R., Gouw, A. M., & Dang, C. V. (2010). Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proceedings of the National Academy of Sciences, 107*(5), 2037-2042.

Lei, K., Bai, H., Wei, Z., Xie, C., Wang, J., Li, J., & Chen, Q. (2018). The mechanism and function of circular RNAs in human diseases. *Experimental Cell Research, 368*(2), 147-158.

Lheureux, S., Gourley, C., Vergote, I., & Oza, A. M. (2019). Epithelial ovarian cancer. *The Lancet, 393*(10177), 1240-1253.

Liu , Y., Wang, Y., Fu, X., & Lu, Z. (2018). Long non-coding RNA NEAT1 promoted ovarian cancer cells' metastasis through regulation of miR-382-3p/ROCK1 axial. *Cancer science, 109*(7), 2188-2198.

Liu, X., Huang, H., Remmers, N., & Hollingsworth, M. A. (2014). Loss of E-cadherin and epithelial to mesenchymal transition is not required for cell motility in tissues or for metastasis. *Tissue barriers, 2*(4), e969112.

Liu, Y., Jin, Z.-R., Huang , X., Che, Y.-C., & Liu, Q. (2020). Identification of Spindle and Kinetochore-Associated Family Genes as Therapeutic Targets and Prognostic Biomarkers in Pancreas Ductal Adenocarcinoma Microenvironment. *Frontiers in oncology, 10*, 2084.

Loh , C. Y., Chai , J. Y., Tang , T. F., Wong , W. F., Sethi , G., Shanmugam , M. K., . . . Looi , C. (2019). The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. *Cells, 8*(10), 1118.

Lu, W., & Kang , Y. (2019). Epithelial-Mesenchymal Plasticity in Cancer Progression and Metastasis. *Developmental cell, 49*(3), 361-374.

MASSAD, L., HUNTER, V., CHERYL A, S., CLARKE-PEARSON, D., & CREASMAN, W. (1991). Epithelial Ovarian Tumors of Low Malignant Potential. *Obstetrics & Gynecology, 78*(6), 1027-1032.

Medici, D., Hay, E. D., & Olsen, B. R. (2008). Snail and Slug promote epithelial-mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor-beta3. *Molecular biology of the cell, 19*(11), 4875-87.

Mo, W. L., Jiang, J. T., Zhang, L., Lu, Q. C., Li, J., Gu, W. D., . . . Wang, H. T. (2020). Circular RNA hsa_circ_0000467 Promotes the Development of Gastric Cancer by Competitively Binding to MicroRNA miR-326-3p. *BioMed research international, 2020*, 4030826.

O'Brien , J., Hayder, H., Zayed , Y., & Peng , C. (2018). Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Frontiers in endocrinology, 9*, 402.

Ouellet, V., Provencher, D. M., Maugard, C. M., Page, C., Ren, F., Lussier, C., . . . Masson , A. (2005). Discrimination between serous low malignant potential and invasive epithelial ovarian tumors using molecular profiling. *Oncogene, 24*, 4672–4687.

Qu, S., Yang, X., Li, X., Wang, J., Gao, Y., Shang, R., . . . Li, H. (2015). Circular RNA: A new star of noncoding RNAs. *Cancer letters, 365*(2), 141-148.

Ramos, F., Wons, L., Cavalli,, I., & Ribeiro,, E. (2017). Epithelial-mesenchymal transition in cancer: An overview. *Integrative Cancer Science and Therapeutics*.

Rosen, D. G., Yang, G., Liu, G., Uribe, I. M., Chang, B., Xiao, X., . . . Liu , J. (2009). Ovarian cancer: pathology, biology, and disease models. *Frontiers in bioscience (Landmark edition), 14*, 2089-2102.

Son, H., & Moon, A. (2010). Epithelial-mesenchymal Transition and Cell Invasion. *Toxicological Research, 26*, 245-252.

Stewart, C., Ralyea , C., & Lockwood , S. (2019). Ovarian Cancer: An Integrated Review. *Seminars in oncology nursing, 35*(2), 151–156.

Storey, D. J., Rush, R., Stewart, M., Rye , T., Nafussi, A., Williams , A. R., . . . Gabra, H. (2008). Endometrioid epithelial ovarian cancer : 20 years of prospectively collected data from a single center. *Cancer, 112*(10), 2211-20.

Su, M., Xiao, Y., Ma, J., Tang, Y., Tian, B., Zhang, Y., . . . Wang, W. (2019). Circular RNAs in Cancer: emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers. *Molecular cancer, 18*(1), 1-17.

Tarhriz, V., Bandehpour, M., Dastmalchi, S., Ouladsahebmadarek, E., Zarredar, H., & Eyvazi, S. (2018). Overview of CD24 as a new molecular marker in ovarian cancer. *Journal of Cellular Physiology, 234*, 2134 - 2142.

Tian , H., Cao, Y.-X., Zhang , X.-S., Liao, W.-P., Yi, Y.-H., Lian , J., . . . Sun, F. (2013). The targeting and functions of miRNA-383 are mediated by FMRP during spermatogenesis. *Cell Death & Disease volume, 4*.

Usman , S., Waseem , N. H., Nguyen , T. K., Mohsin, S., Jamal , A., Teh , M.-T., & Waseem , A. (2021). Vimentin Is at the Heart of Epithelial Mesenchymal Transition (EMT) Mediated Metastasis. *13*(19), 4985.

Vang, R., Shih, I.-M., & Kurman, R. (2009). Ovarian Low-grade and High-grade Serous Carcinoma. *Advances in Anatomic Pathology, 16*(5), 267-282.

Wang, X., Xu, D., Pei, X., Zhang, Y., Zhang, Y., Gu, Y., & Li, Y. (2020). CircSKA3 Modulates FOXM1 to Facilitate Cell Proliferation, Migration, and Invasion While Confine Apoptosis in Medulloblastoma via miR-383-5p. *Cancer management and research, 12*, 13415–13426.

Wang, Y., Mang, M., Wang, Y., Wang, L., Klein , R., Kong, B., & Zheng, W. (2015). Tubal origin of ovarian endometriosis and clear cell and endometrioid carcinoma. *American journal of cancer research, 5*(3), 869–879.

Wang, S., Chen, X., & Qiao, T. (2021). Long non-coding RNA MIR4435-2HG promotes the progression of head and neck squamous cell carcinoma by regulating the miR-383-5p/RBM3 axis. *Oncology reports, 45*(6), 99.

Wang, S., Chen, X., & Qiao, T. (2021). Long non-coding RNA MIR4435-2HG promotes the progression of head and neck squamous cell carcinoma by regulating the miR-383-5p/RBM3 axis. *Oncology reports, 45*(6), 99.

Wu, N., Yuan, Z., Du, K. Y., Fang, L., Lyu, J., Zhang, C., . . . Yang, B. B. (2019). Translation of yes-associated protein (YAP) was antagonized by its circular RNA via suppressing the assembly of the translation initiation machinery. *Cell Death & Differentiation, 26*(12), 2758–2773.

Xie , L., & Pan, Z. (2021). Circular RNA circ_0000467 regulates colorectal cancer development via miR-382-5p/EN2 axis. *Bioengineered, 12*(1), 886–897.

Xu, T., Li, Y., Zhu, N., Su, Y., Li, J., & Ke, K. (2022). circSKA3 acts as a sponge of miR-6796-5p to be associated with outcomes of ischemic stroke by regulating matrix metalloproteinase 9 expression. *European journal of neurology, 29*(2), 486–495.

Yang, X., Mei, J., Wang, H., Gu, D., Ding, J., & Liu, C. (2020). The emerging roles of circular RNAs in ovarian cancer. *Cancer Cell International, 20*(1), 1-11.

Zhao, J., Huang, X., Xu, Z., Dai, J., He, H., Zhu, Y., & Wang, H. (2017). LDHA promotes tumor metastasis by facilitating epithelial-mesenchymal transition in renal cell carcinoma. *MOLECULAR MEDICINE REPORTS, 16*, 8335-8344.

Zhao, X., Guan, J., & Luo, M. (2021). Circ-SKA3 upregulates ID3 expression by decoying miR-326 to accelerate the development of medulloblastoma. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia, 86*, 87-96.

Zheng, M., Jiang, Y.-p., Chen, W., Li, K.-d., Liu, X., Gao, S.-y., . . . Liang, X.-h. (2015). Snail and Slug collaborate on EMT and tumor metastasis through miR-101-mediated EZH2 axis in oral tongue squamous cell carcinoma. *Oncotarget, 6*(9).

Zhong, Y., Zhuang, Z., Mo, P., Lin, M., Gong, J., Huang, J., . . . Huang, M. (2021). Overexpression of SKA3 correlates with poor prognosis in female early breast cancer. *PeerJ, 9*, e12506.

Zhu, W., Ma, L., Qian, J., Xu, J., Xu, T., Pang, L., . . . Zhou, J. (2018). The molecular mechanism and clinical significance of LDHA in HER2-mediated progression of gastric cancer. *Am J Transl Res, 10*(7), 2055-2067.

APPENDICES

Appendix A

Table 1: Clinical samples in this project

Tumor Samples from Ottawa Ovarian Cancer Tissue Bank		
Sample ID	Stage	Histological Subtypes
H1	IIIC	Serous High Grade
H2	IIC	Serous High Grade
H3	IIC	Serous High Grade
H4	IIC	Serous High Grade
H5	IIC	Serous High Grade
H6	IIIC	Serous High Grade
H7	IIIB	Serous High Grade
H8	III	Serous High Grade
H9	IIIC	Serous High Grade
H10	IIIB	Serous High Grade
L1	IC	LMP
L2	IA	LMP
L3	III	LMP
L4	Not identified	LMP
L5	Not identified	LMP
L6	Not identified	LMP
L7	Not identified	LMP
L8	IIIA	LMP
L9	Not identified	LMP
L10	Not identified	LMP
L11	III	LMP