REVIEW

Bali Medical Journal (*Bali MedJ*) 2023, Volume 12, Number 1: 1001-1008
P-ISSN.2089-1180, E-ISSN: 2302-2914

The presence of stem cells in ovarian cancer: a review

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Received: 2022-12-26
Accepted: 2023-03-02
Published: 2023-03-24

ABSTRACT

Ovarian cancer is one of the deadly gynecological malignancies. The disease has usually difficult to detect at the early stage as there are only a few specific symptoms and not many screening methods, thus patients usually come in an advanced and metastatic stage of the disease. Initial therapy for the disease includes surgery to remove the tumor, then followed by chemotherapy. This therapy shows a promising result at first until some sign of reoccurrence starts to show. One of the main reasons is thought to be the presence of a small subset of cells called cancer stem cells (CSCs). These cells may escape from the initial therapy, and due to the CSCs' ability to self-renew, grow into various lineages, and multiply widely. There are multiple efforts done to isolate and identify this subset cell, such as identifying the specific antigen in the cell surface, using Side Population, the ability of CSCs to form floating spheres in serum-free media, and the level of ALDH expression. Such methods have been performed together to identify purely cancer stem cells from the malignancy. The ability of the cell to produce new tumors in immuno-deficient animals, however, is the gold standard for identifying CSC, according to the current study. This review will discuss several methods that have been used to isolate cancer stem cells and prove the stemness of these cells by injecting them into immuno-deficient mice. Cancer stem cells also can avoid the effect of chemotherapy. Several things contribute to this ability. This review there will discuss methods that have been several things that make it possible to be able to escape chemotherapy drugs. By understanding these mechanisms, hopefully, new therapy modalities will be developed and specifically targeted, so that can be used to treat malignancies more effectively.

Keywords: ovarian cancer, cancer stem cells, chemoresistance.
Cite This Article: Anggraeni, T.D., Thiono, J., Pratiwi, I.W., Hariadi, A., Kispa, T.D., Kekalih, A., Askandar, B., Lisnawati., Prijanti, A.R., Aziz, M.F., Winarto, H., Tan, M.I., Andrijono. 2023. The presence of stem cells in ovarian cancer: a review. *Bali Medical Journal* 12(1): 1001-1008. DOI: 10.15562/bmj.v12i1.4011

INTRODUCTION

With over 20,000 new cases recorded and more than 14,000 death cases in 2016 by the Centre for Disease Control and Prevention, ovarian cancer is the tenth most prevalent and the fifth leading cause of cancer death in women.[1] Based on its cellular composition, ovarian cancer is divided into three histological types: stromal, germ cell, and epithelial ovarian carcinoma (EOC). Ninety percent (90%) of ovarian cancer cases are EOC, which has a high mortality rate that is a result of both the disease's insidious onset with vague symptoms and the paucity of effective screening tools. EOC can be divided into five principal histotypes: high-grade serous (70%), endometrioid (10%), clear cell (10%), mucinous (3%), and low-grade serous carcinomas (<5%).[2-5]

It was estimated that 80% of patients have advanced disease when they receive their initial diagnosis. It was anticipated that they would remain asymptomatic in the early stages of the disease because there are currently few biomarkers with sufficient sensitivity to detect the disease at its earliest stages, which contributes to the disease's high fatality rate.[6,7] The 2014 revision to the 1973 publication of the FIGO staging system. Surgery alone is sufficient as a treatment for stage I EOC (disease is contained to one or both ovaries), which is typically linked with favorable survival rates. Stage II tumors involve either one or both ovaries and have migrated to the uterus, fallopian tubes, and pelvic region. One or both ovaries are involved in stage III EOC tumors, and they have migrated to the peritoneum, lymph nodes, and/or other locations outside the pelvis. Stage IV is characterized by distant metastases that impact the lungs, bones, lymph nodes, liver, spleen, and/or other organs or tissues outside the peritoneal cavity. The five-year survival rate for ovarian cancer is up to 92% if it is found and treated in the early stages.[8]

Unfortunately, less than 20% of ovarian cancer is detected in the early stage, making the five-year survival rate drop to less than 30% hence contributing to the highest mortality among all gynecologic malignancies. This is partly because ovarian cancer has few early or distinct symptoms, which frequently overlap with those of other prevalent gastrointestinal, genitourinary, and gynecological disorders and have not yet been demonstrated to be helpful for early diagnosis.[3,6,8] The standard treatment of ovarian cancer is cytoreductive surgery, aiming to debulk the gross tumor, followed by chemotherapy.[9] However, in about 60-80% of cases, patients develop resistance to the

drug and eventually experience relapse within 6 months to 2 years of treatment.

The recurrent cancer is described as more malignant, fast-spreading, and resistant to previous drug treatment.[3,7] This is thought to be caused by a small population of cancer called cancer stem cells (CSC) that can renew themselves and undergo differentiation. This subpopulation of cells takes up less than 5 percent of the whole ovarian cancer population, which can generate new tumors in appropriate hosts. However, for a progenitor cell to regain the capacity for self-renewal, it must experience a mutation.[10] It also provides a potential explanation for why many treatments appear to be successful at first but later fail. The rarer and notably unseen populations of CSCs may persist after the tumor first seems to have been removed, but they later resurface.[11]

The methods used to collect ovarian cancer stem cells, the characteristics of chemoresistance held by cancer stem cells, and the treatment approaches taken to eradicate cancer stem cells are all covered in this overview of the current literature.

CANCER STEM CELLS

The number of recurrence cases in malignancies that previously responded to treatment is getting higher suspected due to the existence of a small group of cells that resides in the tumor called cancer stem cells.[12] Cancer stem cells are thought to be the mutation of normal stem cells, as it shows some abilities that are also found in normal stem cells. These qualities include the capacity for self-renewal, the ability to create numerous lineages, and the ability to proliferate widely. Additionally, altered progenitor cells can likely give rise to cancer stem cells. Such progenitors go through mutations that give them the capacity to self-renew again.[13] These cells habitually remain dormant, making the CSCs less sensitive to treatment compared to the bulk tumor they generate. Furthermore, genomic and epigenomic changes also contributed to these treatment-resistant properties, along with the interactions of these cells with their microenvironment.[12]

CSC was first identified in acute myelogenous leukemia (AML) in 1994 by Lapidot et al. Based on the distinct expression of the cell surface markers CD34 and CD38, the study isolated a subpopulation of cells. Immunodeficient mice can develop leukemia from AML cells that express CD34+/CD38-, even though they only make up a small portion of the total leukemic cell population.[14] Later in 2003, the first CSCs isolated from the solid tumor were identified by Al. Hajj in breast cancer.[15] Ever since then, CSCs have been identified in other kinds of cancer, such as brain, colon, head and neck, pancreatic, liver, lung, prostate, and ovaries.[14,16]

Several theories propose the development of malignant tumors. The hierarchy or CSC model and the stochastic model, often known as the clonal evolution (CE) model, are two of the most well-known theories.[17-19] The somatic mutation that occurs repeatedly is the conventional explanation for how cancer develops and spreads. According to the theory of the stochastic model, repetitive mutations result in the loss of particular tissue traits, which causes the organism to regress to its original phenotype. According to this theory, each tumor cell is physiologically comparable at the outset and can start, support, and promote tumor growth. These functions depend on both extrinsic and intrinsic elements from the tumor microenvironment and signaling system. Later, these cells experienced genetic mutation, becoming cancer cells. Increased proliferative potential, tumor formation capacity, tumor aggressiveness, treatment resistance, and other capabilities are among these cells' traits. The tumor-initiating activity, however, cannot be extracted using any cell-sorting techniques according to this paradigm. Examples of this technique can also be seen in both solid and non-solid tumors, including lymphoblastic leukemias, lung adenocarcinomas, and colorectal cancer.[17-19]

In contrast to the stochastic model, the hierarchical model or CSC model describes a small number of self-renewal subpopulation cells known as tumor-initiating cells (TICs) that possess a stemness profile and are ultimately responsible for the development of tumors. These cells can repopulate the clonal tumor with the same heterogeneity as previous cancer. They are responsible for the growth of the clonal tumor, which eventually contributes to the heterogeneity of cancer. Within the tumor, TICs, subsequently also known as CSCs, can self-renew and differentiate into several cell types, each with unique capabilities and phenotypes.[17-19]

The hierarchical model differs from the stochastic model in that it provides a useful representation of tumor relapse in cancer patients when not all cancer cells and CSC were effectively targeted during therapeutic approaches. The intrinsic characteristics that can be exploited to distinguish and isolate CSCs from the entire tumor cell population could also be predicted by the CSC model. These two models, however, might offer more details for examining tumor cells. De-differentiation processes are used to illustrate a potential relationship between the two models, allowing for interconversions and correlation. As a result, it's plausible that the tumor cells developed using the stochastic model and then dedifferentiated into stem-like cells.[17,18]

Ovarian Cancer Stem Cells

Female reproductive tract tissue constantly undergoes renewal as a result of an injury caused by the ovulation cycle that occurs regularly every month in the reproductive years. The epithelium in the ovarian surface at the apex ruptures during ovulation, creating a stigma wound that releases the oocyte and is then quickly healed. A select few potent tissue-specific stem cells work to maintain this balance. Fimbria sweeps the ovarian surface during ovulation to gather the oocyte discharged from the follicle for fertilization. Fimbriae are exposed to the pro-inflammatory repeatedly during the follicular rupture, imposing an obligate requirement for resident stem cells to facilitate the cellular replenishment.[20,21]

The outermost layer of the ovary is covered by a single layer of cells called the ovarian surface epithelium (OSE), which is made up of uncommitted mesothelial cells that are flat to cuboidal in shape. In OSE, the process of injury and rupture confers plasticity by encouraging the

expression of genes required for tissue remodeling in both the epithelial and mesenchymal tissues. Reactive oxygen species and cytokines generated during ovulation expose the epithelium to DNA damage, which over time may lead to malignant transformation. Due to the liberated epithelial cells getting trapped in the ovarian stroma due to the OSE repair process being impaired, inclusion cysts may also develop. The cysts undergo metaplasia when they are exposed to hormones that encourage growth.[20,21]

ISOLATION OF CANCER STEM CELLS

In terms of membrane transport, DNA repair, and the capacity to control self-renewal and differentiate in response to mutation and outside stimulus, cancer stem cells are similar to normal stem cells. These characteristics enable them to withstand therapies intended to target high cell division rates. The identification of CSC markers and their function in the mechanism of carcinogenesis plays a crucial role in understanding tumor cell behavior, to understanding further the new therapeutic modalities because this subgroup of tumor cells only makes up less than 5% of the total population.[22,23] There are markers specific to identifying and isolating cancer stem cells and normal stem cells. While the specific markers are thought to be the most reliable method, the utilization of enzymatic treatment of cell suspensions may modify surface marker expression.[24]

Attempt to identify ovarian cancer stem cells specific markers have been made using an injected mouse that has been genetically modified with ovarian cancer cells, some human ovarian cancer lines, and samples obtained from patient's ascites.[25] There are several methods used to detect and isolate cancer stem cells. These techniques include the ability to form a floating sphere in a serum-free medium, the ability to distinguish these cells from non-CSCs using cell sorting techniques like flow cytometry and MACS using CSC-specific cell surface markers, the detection of Side Population (SP) by effluxion of the Hoechst 33342 dye-stain, the measurement of ALDH (Aldehyde dehydrogenase) activity within the cell, and therapy resistance assays. Each of the methods have its advantages and disadvantages, therefore it is really important to use multiple methods to specifically identify cancer stem cells. Recent investigations have established that xenotransplantation into immunodeficient mice is the gold standard for identifying CSC.[24,26]

FACS AND MACS

CSCs can be specifically isolated from non-CSCs by sorting these cells using CSC-specific cell surfaces markers, such as flow cytometry and MACS (magnetic-activated cell sorting). A specific monoclonal antibody is attached to super-paramagnetic and biodegradable microbeads in MACS, which enables the enrichment of cells that express the target antigen. FACS (fluorescent-activated cell sorting) is an alternative separation technique that may be used to separate cells according to their emission wavelengths by sorting CSC using fluorochromes in direct or indirect immune fluorescence labeling. Either primary or secondary antibodies can be directly conjugated with the fluorochrome. MACS is typically less difficult than FACS and requires less sophisticated equipment. MACS, however, is unable to simultaneously isolate cells using numerous markers.[27] Another study done by Curley et al showed positive results related to the identification of cancer stem cells using the FACS method with CD133+ markers in mice that were previously injected with ovarian cancer cells.[28] Ferrandina et al. used flow cytometry and monoclonal antibodies against CD133-1 and CD133-2 to further demonstrate the existence of CD133+ cells in the metastatic tumor and ovarian cancer. In a variety of malignancies, including brain and prostate cancers, CD133+ cells have been identified as cancer stem cells due to their prodigious ability to proliferate. Following MACS purification, these cells had a CD133+ cell population purity range of 85% to 95%.[29]

SPHERE-FORMING IN SERUM-FREE MEDIUM

Recent studies have demonstrated the ability to grow CSCs from epithelial organs into floating spheres in serum-free media. The self-renewal, overexpression of genes that contribute to the stemness of the cell, increased ability in tumorigenesis, and improved treatment resistance is characteristics that can be predicted from CSCs.[30] Liu et al. collected samples from primary ovarian tumors and suspended them in serum-free media and later formed non-adherent spheres. The spheres were split up into individual cells which eventually gave rise to a secondary sphere 7 days after plating, which was confirmed for having stem cell phenotype by the expression of Nanog, Oct4, sox2, nestin, CD133, CD177, and ABCG2, and showed enhanced drug resistance, specifically to cisplatin and paclitaxel. The experiment showed that the percentage of CD133+/CD117+ in 57,35% of sphere cells. After injecting 500 sphere cells injected subcutaneously into nude mice, the mice developed tumors with the exact phenotype as the original tumor with an average of 69 days tumor latency.[30] Liao et al. developed sphere-like cells from human ovarian cancer lines and showed that sphere cells were also successfully developed, having similar stem cell features as the ones established from primary tissue culture.[31] He et al. also isolated the potential CSCs from six specimens of high-grade serous ovarian carcinoma. Sphere colonies were formed within one week of culture. Later the cell from the first sphere give rise to the second sphere and later formed tertiary spheres. Oct-4, Nanog, Sox-2, Bmi-1, and Nestin were all expressed at increased quantities in these spherical cells, indicating the stemness phenotype. When injected subcutaneously into female NOD/SCID mice, spherical cells also exhibited better resistance to cisplatin and paclitaxel and were capable of producing new tumors faster and with fewer injections than adherent cells.[32] These findings demonstrate the significant tumorigenicity of sphere-forming cells and this subpopulation of cells may reside within ovarian neoplasm, thus playing a critical role in performing the quality of CSCs such as tumorigenesis, metastasis, and chemotherapy resistance. The spheroid cells are thought to be responsible for this ability by increasing anaerobic glycolysis and the pentose cycle while decreasing the rerouting of glucose for anabolic reasons,

Table 1. Summarize several studies conducted to isolate cancer stem cells (CSCs) using several different methods.

References	Methods	Origin of Cells	Results
Curley et al. (2009)[28] Ferradina et al. (2008)[29]	Flow Cytometry	Primary ovarian tumor	CD133+ in ovarian cancer and metastasized tumor
Liu et al. (2012)[30] Liao et al. (2014)[31] He et al. (2014)[32]	Sphere formation in serum-free medium	Primary ovarian tumor RP-OVI7534 cell line OV2774 cell line	500-10,000 sphere cells were generated Time needed to induce tumors varies between 31-100 days
Dou et al. (2011)[27] Vathipadiekal et al. (2012)[16]	Side Population	Primary ovarian tumor SKOV3 cell line A224 cell line OVCAR-3 cell line UCI-107 cell line	Tumors were generated in 3 out of 3 xenograft mice Time needed to introduce tumors varies between 8-23 weeks
Yasuda et al. (2013)[35]	ALDH activity	MCAS cell line HTBoA cell line OVCAR-3 cell line OVSAHO cell line Human endometrial carcinoma cells	100-1000 $ALDH^{Br}$ cells were sorted

thereby making sphere cells able to survive and metastasize.

SIDE POPULATION (SP)

Another method that is commonly used is the recognition of Side Population (SP). This method takes advantage of CSC properties to actively spit xenobiotics out of them, commonly defined as efflux. This property is facilitated by the expressed protein in membrane cells, the ABC family protein. SP cells can efflux the dye through their ABC transporters when Hoechst 33342 dye-staining is used. The dye-negative fraction is called SP, and later will be sorted using FACS.[24,33] Several studies have used this method and showed the satisfactory result.

In 2011 a study conducted by Dou et al. performed sorting of SP cells by using an A2780 cell line. They demonstrated that the SP cells which were initially stained with Hoechst 33342 and later sorted using FACS also had the features of cancer stem cells, such as ABCG2 molecule phenotype cells, and colony forming. The tumorigenic experiment showed that four out of six nude mice injected with SP cells generated tumors in 23 days, meanwhile, only one out of six nude mice was able to generate tumors in 60 days in non-SP cells.[27] Another experiment conducted by Vathipadiekal et al. using fresh ascites from four ovarian cancer cell lines showed that SP cells were detected in all cell lines evaluated, namely SKOV3 (1,54%), A224 (1,02%), OVCAR-3 (0,08%), and UCI-107 (0,12%). The authenticity of these cells was later also confirmed by qRT-PCR, immunofluorescence, and western blotting methods. The xenograft experiment also demonstrated that tumor growth was observed in three out of three animals that had received injections of SP cells eight weeks following the injection, but not in mice that had received injections of non-SP cells at the same time.[16]

ALDH ACTIVITY

Identifying cancer stem-like cells may also utilize the activity of Aldehyde dehydrogenase (ALDH). For example, the study conducted by He et al. using floating-sphere formations showed that the spheres were able to generate new tumors in nude mice, were showing the properties of cancer stem cells, such as upregulation of stem cell genes, self-renewal, ability to differentiate to various cells, and showing high activity of ALDH.[32] Another experiment done by Yasuda et al. using human ovarian cancer cell lines (MCAS, HTBoA, OVCAR3, and OVSAHO) and human endometrial carcinoma cells identified cancer stem cell population using side population analyses, and later tested for the ALDH activity, categorized as $ALDH^{Br}$ for the cell that appeared brightly in fluorescence. Later, the NOD/SCID mice, which were injected with as few as 10^3 and 10^2 $SP/ALDH^{Br}$ cells, also generated tumors. The mice injected with $SP/ALDH^{Br}$ cells generate tumors in a shorter amount of time, compared to only SP cells and non-SP cells. This finding aligns with the understanding that $SP/ALDH^{Br}$ cells are enriched with cancer stem cell properties.[34]

MECHANISMS OF CHEMORESISTANCE IN CANCER STEM CELLS

Chemotherapy is one of the modalities used to treat malignancy. However, more than 90% of cases eventually recur, and it is thought to be caused by the chemoresistance properties of the cancer stem cells that reside in the cancer tissue. Chemoresistance may be a trait that tumors naturally possess or it may develop as a result of the treatment. Acquired chemoresistance may cause the tumor not only to develop resistance to the first chemotherapy drug but also to develop resistance to other medications with various modes of action. Several mechanisms are thought to contribute to this property, including certain oncogenes that promote tumor initiation and growth. This section will discuss several properties that cancer stem cells possessed regarding their role in contributing to chemoresistance.[36-39]

DRUG EFFLUX

The capacity of the cell to efflux Hoechst 33342 dye via the ATP-binding cassette (ABC) transporters is one of the ways used

to identify cancer stem cells. In the plasma membrane of cells, ABC transporters are typically found, and in a healthy state, they work to shield the cells from damaging toxins and xenobiotics. The efflux capability of the cell is influenced by various types of ABC transporters, including P-glycoprotein (MDR1), ABCB1, ABCC1, and ABCG2. MDR1 is frequently present in the placenta, kidney, adrenal glands, and capillary blood vessels of the brain. They present in low levels and are responsible for preventing toxins from entering sensitive organs like the brain or fetus. ABCG2 is also found in the blood-brain barrier, mammary glands milk ducts, and hematopoietic stem cells. The alteration in these transporters to efflux drugs has been demonstrated *in vitro* to efflux chemotherapeutic agents widely, such as doxorubicin, etoposide, vinblastine, and paclitaxel. For example, doxorubicin is effluxed by both ABCG2 and ABCB1. Other drugs, like paclitaxel, are only effluxed by MDR1 but not by ABCG2. Therefore, these MDR1-expressing cells are more resistant to paclitaxel, but not to ABCG2-specific medications like SN-38.[36,37,40]

Chemoresistance is positively linked with increased expression of ABC transporters. For instance, doxorubicin-resistant breast cancer stem cells were shown to have enhanced ABC1 expression. Additional names for these cells include SP (side population) of tumor cells.[37,40] SP cells are also proven to be able to generate new tumors in immuno-deficient animals, thus confirming the gold standard for cancer stem cells.[41] One of the methods used to overcome this mechanism of chemoresistance is using the specific inhibitors of ABC transporters. There are several drugs still in the process of a clinical trial.[37]

ALDH ACTIVITIES CONTRIBUTING TO CHEMORESISTANCE

A set of enzymes known as aldehyde dehydrogenases (ALDH) catalyze the conversion of aldehydes into carboxylic acids, and an increase in some of its isoforms is associated with the ability of detoxification. Additionally, ALDH showed a tumorigenic potential activity in vivo and is regarded as an accurate marker for locating cancer stem cells. Due to this property, ALDH is considered to play role in contributing to chemoresistance.[2,7]

There are 19 subsets of ALDH.[7] Other than ALDH1, other isoforms of ALDH are located in mitochondria. Although there are many isoforms distributed throughout the body, the kidney and liver are known to express ALDH in the highest quantities. In healthy conditions, ALDH1 permanently transforms retinol into retinoic acid. Due to the high levels of retinoic acid throughout embryonic development and progenitor cell differentiation, it has been discovered that both embryonic multipotent neural stem cells and primitive hematopoietic progenitors exhibit significant ALDH1 activity.[37]

ALDH activity can be measured by Aldefluor, a fluorescent substrate that allows the identification and purification of cells that express ALDH1.[2] By detecting cells with significant ALDH activity, it is first intended to isolate viable hematopoietic stem cells present in the human umbilical cord. In the future, the cells will be referred to as ALDH+ or ALDHbright. Breast cancer and leukemia were found to be tumorigenic cells by strong ALDH activities, and ever since, these cells have successfully started xenograft tumors of different origins. This founding was identified in 1984 when John Hilton closely observed that the level of ALDH in the cyclophosphamide-resistant L1210 leukemic cell line is abnormally high. Since then, it has been shown in several cancer systems that ALDH expression can contribute to cyclophosphamide resistance, leading to the assumption that increased ALDH activity can cause resistance in cancer and cancer stem cells. Several studies have also been conducted to prove the ALDH role in chemoresistance. Acute myeloid leukemic cells with elevated ALDH showed better engraftment in immuno-deficient animals compared to ALDH- cells. Other studies showed that 500 ALDH+ cells were capable to generate xenograft tumors, in contrast to 50,000 ALDH- cells that were unable to form xenograft tumors. Therefore, a therapeutic approach involving ALDH inhibitors might offer practical tools for controlling the chemoresistance property in cancer stem cells.[37]

CHEMORESISTANCE DUE TO QUIESCENCE

Drugs that are used in chemotherapy work as anti-mitotic drugs that target the microtubules essential that are essential for cell division and proliferation. Vinca alkaloids (vincristine, vinblastine, vindesine, and vinorelbine), which stop microtubule polymerization, and taxanes (paclitaxel, docetaxel), which stabilize pre-existing microtubules, are examples of these medications. These medications prevent the creation of the mitotic spindle, which prevents the cell cycle's mitotic phase, thus requiring the cells to be actively cycling and proliferating. However, there is proof that cancer stem cells are dormant, or more quiescent than the non-CSCs. This feature allows them to escape the therapy, thus contributing to their chemoresistance feature. If cancer stem cells manage to re-enter the cell cycle, they will form highly proliferative progenitor cells that can give rise to the bulk tumor. One study demonstrates both *in vivo* that slow-cycling cancer stem cells in various cancer can survive chemotherapy that can reduce bulk tumors, equivalent to two times the concentrations previously used to kill proliferating cells in vitro. These data show that conventional therapy is very ineffective to treat these quiescent cell populations and able to explain the reason for the recurrence of cancer. Even if the bulk tumor is seemed to be eradicated, a single cancer stem cell may survive undetectably with any diagnostic modalities. These cells would also be more likely to give birth to children that are resistant to chemotherapy, so the subsequent tumor would not respond to additional rounds of treatment. Inducing the cells to enter the cell cycle may be one of the options for specifically targeting the cancer stem cells, following a study in a leukemia model, that stimulating the cancer stem cells in a dormant state to divide, may increase the efficacy of cell-cycle chemotherapy.[40,41]

INCREASING DNA DAMAGE REPAIR

DNA damage can occur endogenously through base depurination and deamination or exogenously as a result of

exposure to radiation or other genotoxic chemicals. If the mutation is left unrepaired, the condition is lethal to the cell or may lead to the development of malignancies. However, cells have developed a complex network signaling pathway to repair this damage. The DNA damage response is the capacity to control DNA damage detection and repair to maintain genomic integrity (DDR). DDR mechanism plays a very important role to set the standard for cancer treatment because the treatment targets a very highly proliferative cancer cell. Unfortunately, the improved capacity of cancer stem cells to repair DNA damage is another characteristic of these cells. By turning on the DDR, they might withstand the deadly effects of chemotherapy. Since chemotherapeutic medicine was unable to distinguish between different types of cancer cells, its toxicity in normal tissue caused side effects, which led to restrictions on the dosage and length of treatment. This may be the reason why therapy, despite being helpful, frequently fails to be curative. Additionally, the tumor could become resistant to chemotherapy or radiotherapy, leading to recurrence after the initial therapy.[37,42]

Ataxia Telangiectasia Mutated (ATM) and ATM Rad-3-related are the two main signaling pathways that are activated in response to DNA damage (ATR). 40 They are serine/threonine protein kinases that belong to the phosphatidylinositol 3-kinase-related kinase (PIKK) family. These proteins act as regulators of DDR, cell motility, differentiation, metabolism, and proliferation. By phosphorylating downstream checkpoint kinases 1 (CHK1) and 2 (CHK2), respectively, ATM and ATR function. Later, cell division cycle 25 homolog B (CDC25B) and cell division cycle 25 homolog A are repressively phosphorylated by ATM/CHK2 and ATR/CHK1 (CDC25A). Later, this will hinder the activation of CDKs by members of the CDC25 family and the G1/s and G2/M transitions.[37]

There are many different types of DNA lesions, and one of the most harmful mutations is DNA double-strand breaks (DSBs). Loss of genetic information may cause dangerous mutations and chromosomal rearrangements that ultimately result in the development of cancer. Double strand breaks will cause ATM to activate to induce a cellular response. However, its complicated signals and mechanism for activation are still not fully understood. Checkpoint kinase 2 (CHK2), one of ATM's downstream targets, and the tumor suppressor protein p53 both play a part in the activation of the G1/S cell cycle checkpoint. In addition to the G1/S cell checkpoint, CHK2 and ATM work together to activate the intra-S phase checkpoint.[37]

A single-strand break activates ATR, whereas double-strand breaks trigger ATM. ATR promotes DNA repair, stability, resumption of stalled replication forks, and temporary cell cycle arrest once it is active. The downstream target CHK1 mediates this function. In response to DNA damage, ATMs enforce the intra-S-phase cell cycle checkpoint during normal S-phase progression. By inhibiting the firing of replication origins by facilitating the degradation of Cdc25A through CHK1, it slowed the progression of DNA replication to buy some time to provide resolution of the stress cause. ATR is also a key player in the G2/M cell cycle checkpoint, which prevents cells from entering mitosis too early or in the presence of DNA damage or complex DNA replication. This is accomplished by two mechanisms: CHK1 phosphorylating Cdc25c phosphatase on serine 216 and Cdc25A degradation.[37]

ATM and ATR may be activated by two different types of DNA strand break, but their downstream targets are partially overlapping. For example, ATM can phosphorylate CHK1, which is frequently regarded as the most precise downstream target of ATR. While ATR activates the intra-S-phase and G2/M checkpoints, it is believed that ATM is the main mediator of the G1 cell cycle checkpoint. However, under several circumstances, ATM may help to activate and maintain intra-S-phase and G2/M cell cycle arrest. Additionally, ATM uses CHK2 to mediate Cdc25c phosphorylation. By limiting mitotic entrance through the degradation of Cdc25c phosphatase, both proteins also contribute to the prevention of

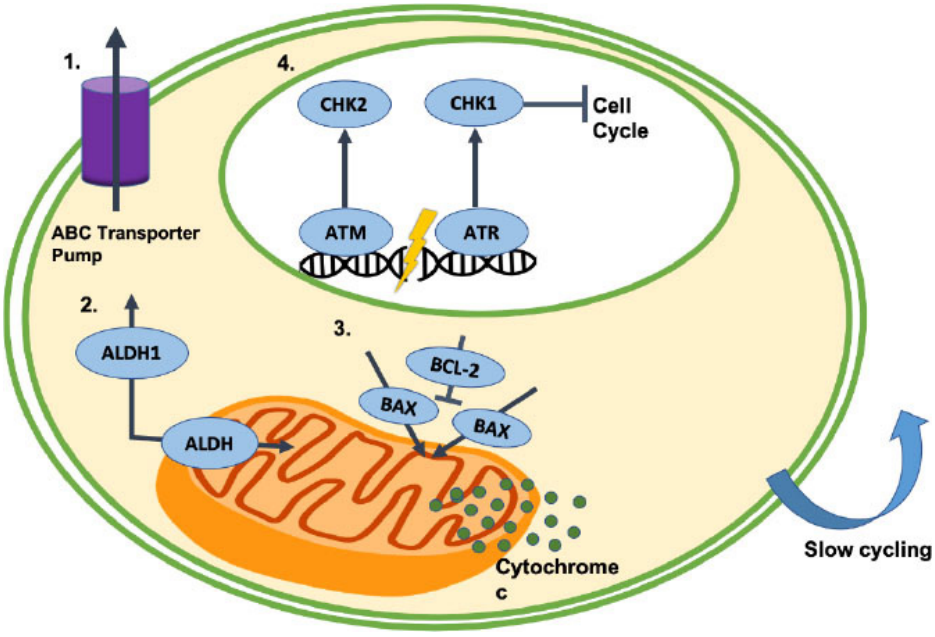

Figure 1. Cancer Stem Cells' Chemoresistance Mechanisms (1). Many different types of chemotherapeutics are effluxed out of cancer cells through ABC transporters. (2). In CSCs that express ALDH, which can localize to both the mitochondria and the cytoplasm, the effectiveness of chemotherapy medicines is diminished. (3). To stop chemotherapy-induced apoptosis, a change in the pro-survival protein BCL-2 attaches to the pro-apoptotic proteins BAX and BAK and prevents the release of cytochrome c from the mitochondria. (4). When CHK2 and CHK1 are activated in response to DNA breaks, ATM and ATR can hinder the cell cycle and encourage DNA repair.

malfunction in telomere activities. By integrating cell cycle progression with DNA repair, they both contribute to modulating cellular responses to a variety of genotoxic stressors and preserving genomic integrity.[37,42]

This mechanism that could protect cancer stem cells has been studied on several occasions. According to one study, after radiation therapy, CD133+ glioma stem cells exhibit higher levels of activated phosphorylation of DNA damage response proteins ATM, CHK1, and CHK2 than CD133- glioma cells. In a different investigation, it was discovered that CD133+ colon cancer stem cells were more resistant to cisplatin than CD133- cells. When compared to CD133- cells, the therapy led to an increase in CHK1 phosphorylation in CD133+ cells.[37]

STRENGTH AND LIMITATIONS

The strength of this article is it reviews and discusses several methods to isolate ovarian cancer stem cells, properties of chemoresistance possessed by cancer stem cells, and therapeutic strategies considered in eliminating cancer stem cells as there has been no article before that reviewed these subjects. However, this article did not include a discussion regarding xenograft as the golden standard of cancer stem cell isolation and this article did not discuss the specific chemoresistance mechanism in ovarian cancer stem cells because it has not yet been discovered.

CONCLUSION

The existence of CSCs in the heterogeneous cellular population in ovarian cancer tissues gives more clues about how cancer may recur in the same patients. Those CSC populations may give rise to new tumors when introduced to a new microenvironment and have the ability of chemo-resistance, giving them more opportunities to survive, repopulate, and metastasize. However, the existence of this subpopulation enables us to identify more therapeutic targets to stop the CSC from self-renewing, differentiating, metastasizing, and also recurring.

ACKNOWLEDGMENTS

Ethical Clearance

This article is cleared under the ethical approval number of 102/UN2/F1/ETIK/2019.

Funding

This review article is funded by Ministry of Research, Technology, and Higher Education (Kementrian Riset, Teknologi, dan Pendidikan Tinggi) Indonesia under the decree number of 6/E/KPT/2019 and the contract agreement number of 1/E1/KP.PTNBH/2019 and 234/PKS/R/UI/2019.

Conflict of Interest

The author reports no conflicts of interest in this work.

Author Contribution

All of the authors contributed equally in this study.

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