G-1: new potential therapeutic option for ovarian cancer

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G-1 was initially developed as a selective agonist for G-protein coupled estrogen receptor 1 (GPER). It has been widely used to investigate the function of GPER in many types of tissues and organs. Our most recent studies unexpectedly found that G-1, in a GPER-independent manner, suppressed ovarian cancer cell proliferation and induced cancer cell death via blocking tubulin polymerization.

Keywords: GPER; G-1; ovarian cancer; cell death; microtubule


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Introduction

Ovarian cancer is the most lethal gynecologic malignancy. Annually, in the United States alone, this cancer affects more than 22,000 women and results in approximately 15,000 deaths. Current treatment is based on the combination of cytoreductive surgery and chemotherapy using taxane and platinum.

The etiology of ovarian cancer remains unknown. Accumulating evidence reveals that estrogen greatly contributes to the development and progression of ovarian cancer [1-2]. It has long been thought that estrogen action is mediated by estrogen receptor α (ERα) and estrogen receptor β (ERβ), which are ligand-dependent transcription factors that regulate downstream gene transcription to exert their cellular functions [3]. However, numerous studies have shown that estrogens also induce cellular actions that are too rapid to be explained by the classic estrogen signaling pathway [4-5]. Indeed, other membrane estrogen receptors such as ER46 and ER36, have been reported to potentially contribute to such rapid estrogen actions [6, 7]. In 2000, Dr. Filardo and colleagues reported that GPR30, a G-protein coupled orphan receptor, is able to mediate rapid estrogen action [8]. Other research groups later confirmed their finding [9, 10]. More recent studies have shown that GPR30 mediates estrogen actions in many organs and tissues. In turn, GPR30 has been since named G-protein coupled estrogen receptor 1 (GPER) [11]. However, studies aimed towards better understanding the mechanisms that underlie how GPER mediates estrogen actions have encountered difficulties. These roadblocks in understanding are primarily due to the fact that 17β-estradiol not only induced activation of GPER, but also other estrogen receptors such as ERα, ERβ, ER46 and ER36.
G-1, a selective GPER agonist, was then developed to distinguish estrogen actions mediated by GPER from that mediated by other estrogen receptors [12]. The primary report showed that G-1 has a high-affinity and selectivity for GPER. G-1 activated neither the traditional estrogen receptors, nor the other 25 G-protein coupled receptors tested [12]. Since then, G-1 has been widely used as a GPER-selective agonist to study the function and mechanism of GPER-mediated estrogen action in many types of tissues and organs [11]. It has been reported that G-1 can stimulate cancer cell proliferation and induce expression of genes associated with cancer cell proliferation [13-15], although the opposite effects have also been observed [16, 17].

In an effort to determine the function of GPER-mediated estrogen actions in the progression of ovarian granulosa cell tumor, we discovered that the knockdown of GPER in KGN cells, a cell line derived from a recurrent ovarian granulosa cell tumor that retains many features of normal granulosa cells, significantly suppressed cell proliferation and reduced cell viability [18]. This result is consistent with previous studies that showed that GPER induced cancer cell proliferation and activation of pathways involved in cell proliferation [13-15]. We then used G-1, the putative GPER agonist, to activate GPER in order to confirm that the activated GPER promotes ovarian tumor cell proliferation. Surprisingly, we found that G-1 treatment did not stimulate KGN cell proliferation. Rather, G-1 suppressed KGN cell proliferation in a concentration-dependent manner. This suppression effect was confirmed in other ovarian cancer cell lines such as SKOV-3, CAOV-3, IGROV-1, COV644, COV362.4 OVCAR-3, and COV434 cells. Because the GPER-agonist G-1 and GPER siRNA similarly affected ovarian cancer cell proliferation, we hypothesized that G-1 suppressed ovarian cancer cell proliferation in a GPER independent manner. This hypothesis was confirmed by the following observations: 1) knockdown of GPER did not affect the action of G-1; 2) blocking the function of GPER using the GPER-selective antagonist G-15 did not affect G-1 suppression of KGN cell proliferation [18, 19]; and 3) G-1 also suppressed proliferation of the GPER-deficient HEK293 cells [10, 18, 20]. These results indicate that G-1 is able to suppress ovarian cancer cell proliferation in a GPER-independent manner. Moreover, we found that G-1 not only suppressed cancer cell proliferation, but also induced cancer cell death in both caspase-dependent and caspase-independent pathways.

We then determined the effect of G-1 on the cell-cycle progression using the flow cytometry and fluorescent immunocytochemistry (ICC). Flow cytometry analysis showed that treatment with G-1 significantly increased cancer cells in the G2/M phase of mitosis. The fluorescent ICC results further indicated that G-1 treatment mainly arrested cancer cells in the prophase of mitosis. Additional experiments showed that treatment with 2 µM of G-1 eliminated cells beyond the prophase of mitosis.

To determine the mechanism of G-1 action, we performed experiments to detect the effect of G-1 treatment on the expression and activation of key proteins and kinases involved in the entry and progression of mitosis. Biochemical analysis showed that G-1 treatment did not affect the expression or activation of mitosis-promoting factors (MPF). Similarly, G-1 treatment did not affect the expression and activation of other critical proteins and kinases governing mitosis entry. Together, this evidence suggests that G-1 does not target the key proteins and kinases governing cell-cycle progression.

During the immunocytochemical analysis, we noticed that ovarian cancer cells treated with G-1 did not form a normal spindle for mitosis. Instead, these cells formed multiple microtubule asters. This observation suggests that G-1 may target microtubule to suppress cancer cell proliferation and induce cancer cell death. Both the analysis of microtubule assembly in living cells and an in vitro tubulin polymerization assay strongly support our hypothesis that G-1 suppresses ovarian cancer cell proliferation and induces cancer cell death via blocking tubulin polymerization, thus leading to disrupted microtubule assembly and subsequent cell death.

Our finding that G-1 suppresses ovarian cancer cell proliferation is important because of its clinical potential. Clinical statistics showed that more than 80% of ovarian cancer patients respond to the standard platinum- and paclitaxel-based chemotherapy [21, 22]. However, the majority of women with advanced ovarian cancer ultimately relapse and develop drug resistance [21, 22]. The standard platinum- and paclitaxel-based chemotherapy has limited effect against these drug-resistant ovarian cancers. The development and use of the new biological agents is largely responsible for improvements in the prognosis of ovarian carcinoma. The finding that G-1 targets microtubules to suppress ovarian cancer cell proliferation and induce cancer cell death makes it a promising anticancer drug and a new potential therapeutic option for recurrent ovarian cancer.

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References

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