HGF/C-Met Expression in Epithelial Ovarian Carcinogenesis and Its Potential as Molecular Targeted Therapy

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ABSTRACT

Background: The Mesenchymal-Epithelial Transition factor (C-Met) is a tyrosine kinase receptor (TKR) that binds to a ligand called Hepatocyte Growth Factor (HGF). Recent studies conducted on patients with epithelial ovarian cancer (EOC) reported that high expression of C-Met was associated with poorer clinico-pathological grading and outcomes. Therefore, this study aimed to further explore the downstream pathway specifically activated when the HGF/C-Met complex was formed, the interplay between C-Met and other molecules, as well as the impact on EOC when these interactions were inhibited through designated molecular targeted therapy.

Methods: The search strategy using the PubMed search engine (https://pubmed.ncbi.nlm.nih.gov/) was conducted on September 21, 2022, with the keywords: “HGF/C-Met and ovarian cancer”. The search resulted in 261 articles, and they were filtered by “published in the last five years,” which yielded 67 articles. These articles then underwent further screening, resulting in 40 articles for analysis. A systematic literature review was conducted to improve the quality of this study. Approximately 150 articles were thoroughly examined and organized using a reference manager, then 15 with the greatest impact and clinical relevance to this study were selected.

Results: The HGF/C-Met complex was found to stimulate signaling pathways linked to the growth of epithelial cells and also caused the phosphorylation of tyrosine residues on other tyrosine receptors. The activation of C-Met affected the downstream pathways involving molecules associated with cell proliferation and survival, such as epidermal growth factor receptor (EGFR), p53, and KRAS. C-Met can be combined with other tyrosine kinase inhibitors in chemotherapy to enhance the initiation of cell death (apoptosis) in cancer cells.

Conclusions: The HGF/C-Met mediated a signaling cascade that played an essential role in the tumorigenesis of ovarian carcinoma and had the potential to be a targeted molecular therapy in EOC.

INTRODUCTION

Ovarian cancer is one of the leading causes of malignancy-related deaths worldwide and the third most common cancer among women in Indonesia [1]. Each year, new cases increase by 4.3%, or approximately 13,310, with the mortality rate rising to 7,842 [2]. Based on histological subtypes, ovarian cancer is divided into two subtypes namely epithelial (90%) and non-epithelial. EOC is a heterogenous cancer with five subtypes including high-grade serous (HGSOC), low-grade serous (LGSOC), endometrioid (EnOC), ovarian clear cell carcinoma (OCCC), and mucin-producing ovarian carcinoma [3].

The pathogenesis is predicated on a dualistic theory, and according to the first hypothesis, ovarian cancer results from the transformation of benign tumor dysplastic cells into malignant tumor carcinoma cells [4]. The second theory proposes that ovarian cancer is caused by genetic mutations transforming normal cells into cancerous ones [5]. Cancer cells often have changes in the cell cycle with many activated oncogene transcripts and oncoproteins involved. These oncoprotein complexes, comprising ligands and receptors, stimulate cell entry into the cell cycle, inhibit apoptosis, and increase the...
biosynthesis of proliferative cellular components. Receptors associated with kinase activity including serine/threonine, tyrosine, lipid, and phosphatase, as well as coupled-protein G and nuclear receptors play a crucial role in regulating the cell cycle. Other related receptors are those that activate transcription factors, such as Notch and Frizzle’s receptors interacting with Wnt ligands. C-Met is an epithelial cell membrane-expressed tyrosine kinase receptor (TKR) that binds to the ligand called hepatocyte growth factor (HGF). This ligand is expressed in connective tissue and smooth muscle cells [6]. It initiates biological processes through paracrine signaling, which is secreted by mesenchymal and fibroblast cells in the stromal membrane and subsequently used by epithelial cells. Under normal conditions, HGF promotes neuron and muscle development, tissue regeneration, wound healing, as well as embryogenesis, which are all controlled by the tumor suppressor gene P53 [7]. This signaling cascade is essential in normal human biological functions.

The interaction between HGF and C-Met leads to dimerization and autophosphorylation of the tyrosine residue. This creates a docking site for intracellular transducer docking, which activates rat sarcoma viral (RAS)-mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and signaling pathway signal transducers (STAT) [8]. The effects of the HGF/C-Met signaling pathway have been observed in various epithelial and mesenchymal cancers. In breast cancer studies, high C-Met expression was reportedly associated with cancer cell aggressiveness and poor patient survival [9], while in ovarian cancer, overexpression was found in 7% to 27% of cases [10]. Several in vitro studies also produced different findings. As ovarian epithelial cells changed their proliferation pattern to become neoplastic, C-Met expression was found to increase significantly [11]. Furthermore, an increased transcript of HGF was discovered in the ascitic fluid of ovarian cancer patients, as well as in cancer cells cultivated from the fluid [12]. The HGF inhibitor, NK4 suppressed the cancerous cell proliferation in culture media [13]. Another study found that C-Met expression was stronger in ovarian cancer cells compared to normal surface epithelial cell lines [14].

C-Met potentially interacts with various proteins, including plexins, CD44, tetraspans, integrins, death receptors, mucins, and RTKs such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and human epidermal growth factor receptor 3 (HER3) [15,16]. These interactions may lead to inhibition or activation, subsequently influencing important cellular processes such as cell survival, proliferation, migration, invasion, oncogenesis, and drug resistance [16,17]. In general, these interactions often lead to synergistic effects on cancer progression, tumorigenesis, and the development of therapy resistance. This implies that C-Met holds great potential as a therapeutic target for various cancers. Numerous inhibitors and antibodies targeting HGF/C-Met have been developed, with some currently undergoing clinical trials [18,19]. Therefore, this literature analysis was conducted to better understand the pathological and prognostic effect of C-Met expression in epithelial ovarian cancer patients as well as its potential for molecular treatment.

METHODS

The main focus of the study

A systematic literature review was conducted using the PICO Formula, where P= Patient with ovarian cancer I= intervention: immunohistochemical examination for HGF and C-Met in different stages of ovarian cancer C= Comparison: Clinicopathological grading and stages in EOC related to HGF/C-Met expression as well as chemotherapy effects, and O= outcome: HGF/C-Met expressed at various level in ovarian cancer, and combination chemotherapy targeting C-Met which can improve cancer cell apoptosis.

Search strategy

Several comprehensive keywords were built into the search term namely “HGF and ovarian cancer” “C-Met and ovarian cancer”, “targeted chemotherapy at HGF/C-Met” and “HGF/C-Met signaling in ovarian cancer”. Original articles containing one of these search terms were retrieved from PubMed, Scopus, Google Scholar, and the Remote Digital Library of Universitas Indonesia.

Selection of eligible studies

Among 150 articles thoroughly examined, 15 were selected in this study as shown in Figure 1. The PRISM flow chart was used to assess the comprehensiveness of studies and determine their inclusion or exclusion.

RESULTS

Among the 150 articles retrieved from the databases, only 15 were eligible for this systematic review as shown in Table 1. In the included articles, several key findings have been identified. Based on the results, HGF/C-Met signaling stimulates a signaling pathway associated with the proliferation of epithelial cells. The activation of the C-Met receptor through the phosphorylation of its tyrosine kinase leads to the subsequent phosphorylation of other proteins, a process known as co-expression. The results also suggested that HGF/C-Met could serve as a molecularly targeted therapy in combination with other chemotherapy.
Figure 1. Systematic review flow diagram using PRISMA flow chart

Electronic database searches: PubMed, Scopus, Proquest, Google Scholar
Institutional Database search: Remote Digital Library Universitas Indonesia
(n = 150)

Titles/abstracts screened (n = 100)
Excluded (n = 35) not relevant to the topic similar articles

Full-text articles assessed for eligibility (n = 65)
Excluded (n = 50) study result is not significant method is not applicable

Studies reported HGF/C-Met expression in ovarian cancer and their potential as targeted therapy

Figure 2. Illustration of C-Met receptor structure [8]

Figure 3. Signaling pathway activated by HGF/C-Met binding [8]
<table>
<thead>
<tr>
<th>No</th>
<th>Author</th>
<th>Country</th>
<th>Study Title</th>
<th>Study Design</th>
<th>Molecular mechanism related to HGF/C-Met signaling</th>
<th>Impact of Ovarian Cancer Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kim HJ, et al. (2019) [17]</td>
<td>South Korea</td>
<td>Humanized anti-HGF (YYB-01) monoclonal Antibody inhibit Ovarian Cancer Progression</td>
<td>Preclinical study</td>
<td>Anti-HGF antibody (YYB-01) binds HGF released by cancer cell</td>
<td>Cancer Cell regression and remission of tumor diameter when combined with paclitaxel in animal model</td>
</tr>
<tr>
<td>2</td>
<td>Lim L, et al. (2019) [18]</td>
<td>China</td>
<td>Clinical significance of c-Met and phospho-c-Met (Tyr1234/1235) in ovarian cancer</td>
<td>Preclinical study</td>
<td>Ovarian clear cell carcinoma and mucinous carcinoma had much higher C-Met expression</td>
<td>C-Met and phospho-C-Met were associated with poor P-F-S, respectively</td>
</tr>
<tr>
<td>3</td>
<td>Kyriakopolous CE, et al. (2017) [19]</td>
<td>USA</td>
<td>A phase I study of tivantinib combination with temsirolimus in patients with advanced solid tumors</td>
<td>Clinical Study</td>
<td>One pt. with ovarian cancer had a confirmed partial response and remained on study for 10 months, a second patient with ovarian cancer had stable disease and remained on study for 6 months and a third pt. with squamous cell carcinoma of the tongue had stable disease and remained on study for 7 months.</td>
<td>The combination of tivantinib and temsirolimus improved progression-free survival in advanced solid tumor patient</td>
</tr>
<tr>
<td>4</td>
<td>Kim WY, et al. (2017) [20]</td>
<td>South Korea</td>
<td>The gene copy number of C-Met has a significant impact on progression-free survival in Korean patients with ovarian carcinoma</td>
<td>Preclinical study</td>
<td>High Polysomy of Chr.7 and gene amplification of MET related to poor progression-free-survival (PFS)</td>
<td>MET gene copy number may serve as a biomarker for poor prognosis ovarian cancer patient</td>
</tr>
<tr>
<td>5</td>
<td>Kim JH, et al. (2016) [21]</td>
<td>South Korea</td>
<td>C-Met as a potential therapeutic target in Ovarian Clear Cell Carcinoma</td>
<td>Preclinical study</td>
<td>Inhibition of C-Met using C-Met inhibitors (SU11274 or crizotinib) significantly decreased the proliferation and increased the apoptosis of OCCC cells.</td>
<td>SU11274 decreased the expression of the p-c-MET proteins and blocked the phosphorylation of downstream proteins Akt and Erk.</td>
</tr>
<tr>
<td>6</td>
<td>Dongdong, et al. (2016) [22]</td>
<td>Japan</td>
<td>The hepatocyte growth factor antagonist NK4 inhibits indoleamine-2, 3-dioxygenase expression via the c-Met-phosphatidylinositol 3-kinase-AKT signaling pathway</td>
<td>Preclinical study</td>
<td>NK-4 activates cancer cell sensitivity to the immune system</td>
<td>NK-4, the antagonist of HGF, enhanced the cancer cell-killing mechanism by the host immune system</td>
</tr>
<tr>
<td>7</td>
<td>Du Y, et al. (2016) [23]</td>
<td>China</td>
<td>Blocking C-Met mediated PARP1 phosphorylation enhances anti-tumor effect of PARP inhibitors</td>
<td>Preclinical study</td>
<td>C-Met associated with and phosphorylates PARP1 in Ovarian Cancer with BRCA mutation</td>
<td>Inhibition of C-Met increased chemosensitivity to PARP1</td>
</tr>
<tr>
<td>8</td>
<td>Isabelle, et al. (2015) [24]</td>
<td>Canada</td>
<td>Ovarian cancer ascites enhance the migration of patient-derived peritoneal mesothelial cells via the C-Met pathway through HGF-dependent and -independent mechanisms</td>
<td>Clinical study</td>
<td>HGF is present at high concentrations in ascite fluid</td>
<td>HGF enhances the migration and invasion ability of ovarian cancer cell</td>
</tr>
</tbody>
</table>
HGF/C-Met signaling induces the activation of pathways related to epithelial cell proliferation, as well as their transition to mesenchyme and vice versa, playing an essential role in the embryonic period [31]. During this period, HGF is produced in the ovarian stromal cells adjacent to the genital ridge, which is vital for ovarian development [32]. Moving on into the reproductive period, HGF is expressed by theca cells to induce ovum maturation. It is also secreted by mesenchymal cells in the stroma ovary to regenerate ruptured ovarian surface epithelium after ovulation [33,34]. The expression in an inactive form is called
pro-HGF, and its activation occurs when there is a breakdown of pro-HGF into HGF by a protease enzyme resembling a urokinase plasminogen activator [35].

The C-Met receptor is encoded by the MET gene, located on the long arm of chromosome 7 between positions 21 and 31 (7q21–q31). This receptor is of the immunoglobulin-like type, possessing both extracellular and transmembrane domains [31]. The transmembrane domain comprises one SEMA, one PSI, four IPT, one transmembrane, one juxta membrane, and one tyrosine kinase domain, as well as one c-terminal (carboxyl terminal) chain [8]. The HGF ligand binds to C-MET in the SEMA domain, while the PSI domain stabilizes the bond. After binding, the intracellular tyrosine kinase domains Tyr-1234 and Tyr-1235 autophosphorylates, followed by autophosphorylation at the c-terminus on Tyr-1349 and Tyr-1356, respectively (Figure 2). The phosphorylation cascades lead to the stimulation of successive signaling pathways as well as the recruitment of intracellular effector molecules such as GRB2, SRC, PI3K, and GAB1 (Figure 3).

**HGF** and C-Met binding initiate communication pathways associated with cell proliferation [36]. There are three major signaling pathways associated with carcinogenesis. The RAS/MAPK/ERK pathway is linked to the synthesis of transcription factors involved in the cell cycle [37], PI3K/AKT is related to angiogenesis and the inhibition of apoptosis [38], while Wnt/β-catenin is involved in proliferation and tumor cell metastases [39]. This article further investigated the three signaling pathways.

**Signaling pathway of RAS/MAPK/ERK**

The MAPK signaling cascade is induced by the activation of the rat sarcoma viral oncogene homolog (RAS) through the C-Met receptor. This activation occurs through the exchange of guanine nucleotides triggered by the binding between SHC and GRB2. Consequently, v-Raf murine sarcoma viral oncogene homolog B1 (RAF) kinases are phosphorylated, leading to the activation of MAPK effector kinase (MEK) and then MAPK. Once activated, MAPK translocates to the nucleus and induces several transcription factors that regulate cell proliferation, motility, and cycle progression [37]. SHP2 is also linked to the cascade through C-Met signaling and the sequestration of GAB1 by SHP2, which contributes to the duration of MAPK phosphorylation [38].

**Signaling pathway of PI3K/AKT/mTOR**

PI3K/AKT is the second signaling pathway initiated by C-Met receptor activation. The P85 subunit can directly or indirectly bind to C-MET through GAB1, thereby attaching to AKT/protein kinase B. This axis regulates the survival response of the cell to C-Met signaling. CRK-mediated phosphorylation of Janus kinase 1 is responsible for the down-regulation of the C-Met receptor (JNK). STAT3 is also phosphorylated, but the underlying mechanism is unclear. C-Met activation causes the recruitment of STAT3 to the tyrosine kinase domain, and their binding causes dimerization, phosphorylation, and nuclear translocation, resulting in the transcription of genes involved in proliferation and invasion [39]. Other studies showed that although C-Met receptors are involved in the process of carcinogenesis, the signaling pathway does not affect the processes of proliferation, invasion, or morphogenesis [40]. This implies that the participation of STAT-3 in C-Met signaling varies depending on the tissue. When activated, phosphatidylinositol 3-kinase (PI3K) converts phosphatidylinositol-4,5-bisphosphate (PIP2) to the second derivative, phosphatidylinositol-3,4-bisphosphate (PI(3)P) [8]. Consequently, proteins with plextin homology (PH) domains are attracted to regions close to the plasma membrane. These proteins incorporate the following signaling downstream effectors: serine/threonine kinase AKT (also known as Protein Kinase B), serine/threonine 3-phosphoinositide dependent protein kinase-1 (PDK1), and mammalian protein target of rapamycin complex 2 (mTORC2) [44]. The simultaneous presence of these proteins triggers the phosphorylation of AKT by PDK1 and mTORC2, both of which can play the role of PDK2, ultimately leading to the full activation of AKT [45]. mTORC2 phosphorylates S473, while PDK1 is responsible for phosphorylating T308. Once activated, AKT inhibits glycogen synthase kinase-3 (GSK3/), a protein that promotes cell proliferation and viability [39]. It also suppresses the transcription factor forkhead box O (FoxOs), which promotes the transcription of proapoptotic genes and cell cycle inhibitors. Additionally, AKT inhibits BAD and caspase-9 [46], two proteins directly related to apoptosis.

**Signaling pathway of Wnt/β-catenin**

The epithelium-to-mesenchymal transition (EMT) is a biological process, wherein epithelial cells lose their ability to adhere to one another and instead acquire the characteristics of mesenchymal cells, including migration and invasion capabilities [41]. This invasive capacity enables cells to penetrate the basement membrane, ultimately producing metastasis in ovarian cancer. Previous studies examining the characteristics of the invasive pattern in ovarian cancer stated that EMT activation was an essential phenotype of malignant tumors, particularly high-grade ovarian carcinoma. Several evidence indicated that E-cadherin and β-catenin expression levels are essential in the commencement of EMT. By forming complexes at adherent junctions, E-cadherin helps maintain β-catenin concentrations at low levels in the intracytosol or nucleus. Therefore, decreased levels of E-cadherin are associated with increased expression of β-catenin signaling [42]. HGF/C-Met signaling is linked to β-catenin activation, which is distinct from Wnt/β-catenin. When the C-Met receptor...
binds to HGF, tyrosine residues are phosphorylated at Y1234 and Y1235. This, in turn, causes the phosphorylation of β-catenin on the tyrosine residue Y654 and Y670, culminating in the dissociation of C-Met on the cell membrane [43]. Consequently, HGF/C-Met is linked to β-catenin activation, which occurs independently of the Wnt/β-catenin signaling pathway.

Co-expression between C-Met and other proteins

C-Met receptor activation promotes cell cycle-related signaling, proliferation, and survival [49]. HGF/C-Met activates a signaling pathway through the second mode of tyrosine kinase messenger, which causes the phosphorylation of residues on other tyrosine receptors. This leads to the phosphorylation of other proteins, a phenomenon called co-expression [50,51]. Examples of cross-talk between C-Met signaling and other proteins associated with carcinogenesis were further discussed.

Co-expression between C-Met and EGFR

EGFR and C-Met are frequently co-expressed in malignancies, and both of their respective signaling networks converge on common molecules, including ERK/MAPK and PI3K/AKT. Chemotherapy targeting the EGFR pathway and C-Met activation holds great potential due to the connection between the two pathways. Several studies showed the benefits of therapeutic sequences, and combined treatments [43]. Both C-Met and EGFR are known to play significant roles in the development of ovarian carcinoma. Combinations of their inhibitors have been utilized in different clinical studies, with satisfactory results. C-Met receptor activation reduced growth in OVCAR-5 and SKOV-3 cell cultures, with the effect being dose-dependent in SKOV-3 cell culture [16]. Downstream mediators of TKR such as ERK and PI3K were also activated, as evidenced by the phosphorylation of ERK2 and AKT.

Co-expression C-Met and P53

Tumor suppression proteins are essential in preventing the progression of malignant cells and are commonly mutated in more than half of human malignancies. The majority of these mutations disrupt the ability of p53 to bind DNA, leading to a loss of its normal functions in response to oncogenic stress. These functions include the ability to induce cell cycle arrest and apoptosis. The interplay between these novel functional alterations is closely related to the acquisition of oncogenic functions that contribute to metastasis and invasion. One of the effects of p53 mutation is the dissociation or colony dissemination of epithelial cells, partly caused by the activation of the C-Met receptor through HGF ligands [44]. This mutation also enhances EGFR signaling, although it is less potent than C-Met activation [45]. More importantly, MET-dependent induction of ERK1/2 phosphorylation occurs in these cells.

Co-expression of C-Met and KRAS

Three cellular RAS genes express four homologous proteins namely HRAS, NRAS, KRAS4A, and KRAS4B [58]. KRAS4B, originating from alternative splicing at the C-terminal end, is the most common isoform and is often referred to as KRAS [54]. All RAS isoforms share 85 identical amino acids, including the GDP/GTP bond [55]. The hypervariable region found at the C-terminal end is the primary factor that differentiates each variant from one another [56]. This region is responsible for the localization of proteins in the cell membrane, which is necessary for the occurrence of any biochemical action.

KRAS mutations are often found in low-grade serous-type ovarian carcinoma or mucinous adenocarcinoma. The frequency of type 1 ovarian tumors varies across various case reports, ranging from 30–50%. The RAS-RAF-MEK-ERK signaling pathway, which is part of the MAPK cascade, plays an essential role in cell growth. Aberrations in the regulation are closely associated with cancer progression. KRAS mutations most often occur in this signaling pathway, triggering oncogenes in several cancers [47]. The RAS-MAPK pathway is a key factor in the development of ovarian carcinoma, making it an important therapeutic target. Several clinical trials on low-grade serous ovarian carcinoma now use MEK inhibitors to suppress cancer cell growth, which is controlled by receptor tyrosine kinase (RTK) [48]. Aberrations in RTK lead to the overactivation of subsequent signaling cascades, resulting in uncontrolled cell growth. The crosstalk between C-Met and KRAS occurs through the RAS-MAPK signaling pathway, which functions in the transition from adenoma to carcinoma [5].

The potential of C-Met as a molecular therapeutic target for ovarian carcinoma

The biochemistry and expression patterns of C-Met in a variety of cancers have been investigated, but the results obtained are yet to be incorporated into any chemotherapy regimens to date. The majority of tumors investigated including breast, lung, and colon, metastasize through hematogenous, lymphatic, or direct extension. On the other hand, ovarian cancer metastasizes through peritoneal dissemination and may use different molecular mechanisms. Di Renzo et al. [62] using Western blotting investigated the amount of C-Met expression in fresh tissue obtained from 67 patients identified with papillary thyroid cancer. It was discovered that 21% of the patients had moderate expression, while 7% exhibited strong expression. Sawada et al. [29] found a similar expression pattern using immunohistochemistry, wherein among 138 patients, 27% and 11% had intermediate and strong C-Met expression, respectively. There was also a negative correlation between high C-Met expression and general mortality. A study conducted by Ayhan et al. [30] reported that the expression was linked to both clinicopathological parameters and patient
mortality. High-grade ovarian cancer cases were found to have an increased level of C-Met expression up to 60.9%. Moreover, ovarian carcinomas with stronger expression correlated with a higher histological grade and had paraaortic lymph node metastases. This discovery aligns with previous studies on C-Met expression in clear and renal cell cancer, both of which share a similar histological appearance.

Clinical studies focused on C-Met as a potential therapeutic target can be broken down into two categories: immunotherapy, using monoclonal antibodies (onartuzumab), and small molecule inhibitors. Small molecule inhibitors are targeted therapies focused on specific molecules in the cell and weigh less than 500Da. Currently, 88 small-molecule inhibitors have been approved by FDA [50]. The inhibitors that attach to the tyrosine kinase region of C-Met can be further subdivided into two categories: ATP competitive inhibitors (such as crizotinib) and non-ATP competitive inhibitors (tivantinib).

Monoclonal antibody targeting C-Met

Onartuzumab, an engineered antibody created in Escherichia coli, is a monovalent (one-armed) humanized mAb. It specifically binds to the Sema domain of the MET receptor and inhibits the binding of HGF. This monoclonal antibody prevents MET dimerization through knob-into-hole interaction. By inhibiting the HGF alpha chain and creating a complex with the SEMA-PSI domain of the C-Met receptor, the combination creates a bond that is extremely specific to one another. This allows the antibody to specifically inhibit the interaction between HGF and C-Met. This mechanism operates independently of the receptor dimerization pathway. Clinical trials are currently underway to evaluate the effectiveness of onartuzumab in various cancer types, including lung (adenocarcinoma and squamous cell), colon, breast, stomach, and brain cancers. Phase I studies observed complete responses, and a phase II trial in non-small cell lung cancer (NSCLC) showed an overall survival benefit when onartuzumab was administered in combination with erlotinib [51]. The impact on overall survival (OS) or progression-free survival (PFS) in ovarian cancer trials has not been determined at this point. However, in a study involving triple-negative breast cancer, the addition of onartuzumab did not lead to any improvement in PFS [52].

Small molecule inhibitor targeting C-Met

Crizotinib is an example of a selective C-Met inhibitor and showed promising results in treating cancers associated with C-Met in one clinical study (phase I) carried out in 2014. The study was performed with increased MET amplification. One of its approved clinical applications is for the treatment of ROS1-positive lung cancer. Crizotinib is one of five drugs authorized by the FDA for the treatment of advanced non-small cell lung cancer. Additionally, the number of studies that concentrate on its utilization in conjunction with various other medications is growing. Crizotinib combined with cisplatin caused an arrest in the G2/M phase of the cell cycle as well as apoptosis in ovarian cancer cells, as shown by Huang et al. [65]. The breast cancer cell lines BT474, MCF7, MDA-MB-468, and SKBr3 were utilized by Stanley et al. [66] to characterize the various growth-inhibiting effects produced by the combination of C-Met inhibitors and cytotoxic medicines. According to the observations, the combination of crizotinib and EGFR-TKI had a synergistic effect on MCF7 and MDA-MB-468 cells, but the effect was antagonistic on BT474 and SKBr3 cells. This combination also exhibited a greater impact on breast cancer than single-drug regimens. The sensitivity tests of mitomycin C (MMC) in conjunction with crizotinib against colorectal cancer cell lines showed that the two medicines combined enhanced tumor cell apoptosis with a synergistic impact. Furthermore, Tang et al. [67] discovered that crizotinib suppressed ovarian cancer growth better than C-Met-specific medicines. SU11274, a C-Met inhibitor, reportedly reduced hepatocellular carcinoma cell growth as well as pancreatic cancer cell proliferation. It also hindered the migratory and invasion skills of sorafenib-resistant hepatocellular carcinoma cells, according to Firtina Karagonlar et al. [68]. In vitro studies on ovarian cancer cells showed that this inhibitor reduced cell growth, motility, and invasive activity.

Tivantinib is a non-ATP competitive inhibitor that binds to inactive receptors to halt their activation and potential communication. ARQ197 induces the disturbance of ionic interactions in the catalytic residue, allowing for the direct attachment to the A- and P-loops of phenylalanine. This is accomplished with the assistance of Arg1227, Tyr1230, and other residues [26]. The bioavailability mechanism of this substance has been the subject of debate over the past few years. At first, it was believed that tivantinib exerts its biological effect by directly suppressing the C-Met receptor tyrosine kinase. Subsequent studies showed that the receptor is not necessary to achieve the biochemical effects. Instead, tivantinib suppresses the growth of tumor cells by inducing microtubule depolymerization, a process dependent on the presence of the C-Met receptor. It also stimulates apoptosis and concurrently suppresses tubulin polymerization, elongating the G2/M cell phase, and disturbing tubulin metabolism [19]. According to previous studies, the combination of tivantinib with erlotinib for treatment improved survival and was well-tolerated [69].

Combination chemotherapy targeting C-Met in cancer treatment

Increasing evidence suggests that targeting a single molecule within the intricate pathway of cancer may not be sufficient to prevent downstream events. This
is because alternative cascades can be activated, triggering multiple pathways, and potentially leading to resistance against the initial chemotherapy treatment. According to recent studies, total reliance on single-drug chemotherapy might culminate in the development of drug resistance. The combination of C-Met inhibitor therapy with other treatments has been explored to provide valuable insights into effective therapeutic approaches. Currently, there is a preference for a “horizontal” blockade strategy, where two or more tyrosine kinase inhibitors (TKIs) or other inhibitors are combined to target multiple pathways simultaneously [70]. Several notable examples of successful drug combinations include the simultaneous administration of multiple chemotherapeutic agents.

C-Met and EGFR combination

Abnormal activation of EGFR is associated with various mechanisms such as receptor overexpression, mutation, ligand-dependent receptor dimerization, and ligand-independent activation [71]. Furthermore, it is implicated in the development of different types of cancers in humans [72–74]. The approval of tyrosine kinase inhibitors (TKI) such as erlotinib [69], gefitinib [75], and lapatinib [76] for cancer treatment has fueled the development of numerous novel EGFR inhibitors in the past decade. Gefitinib and erlotinib, classified as first-generation EGFR-TKI, function as reversible inhibitors that competitively bind to ATP [77]. After binding, these inhibitors hinder the autophosphorylation of the tyrosine kinase (TK) domain, thereby preventing the activation of downstream signaling pathways [78]. These first-generation EGFR-TKI showed significant clinical benefits in patients with EGFR mutations. However, over time, patients develop acquired resistance [79], which ultimately limits the long-term effectiveness of these agents.

Moores et al. [80] reported that JNJ-61186372 effectively inhibited the phosphorylation of EGFR as well as C-Met induced by EGF (epidermal growth factor) and HGF, respectively, in a dose-dependent manner. Furthermore, the activity as measured by the half-maximal inhibitory concentration (IC50), was below 100 nmol/L, indicating potent inhibition of both EGFR and C-Met signaling. A lower IC50 value suggests greater effectiveness in inhibiting the target receptors. These findings show that JNJ-61186372 effectively inhibits the activation of both EGFR and C-Met receptors, regardless of the EGFR mutation status, and could have therapeutic potential in cancer treatment by targeting the cross-talk between these receptors.

C-Met and HER-2 combination

HER-2 overexpression is common in approximately 20% to 30% of breast tumors and is associated with reduced disease-free and overall patient survival. Trastuzumab, a humanized monoclonal antibody targeting HER-2, was the first therapy developed to specifically target HER-2-positive breast cancer. It has been shown to reduce the risk of relapse and prolong patient survival. However, resistance, both inherent (pre-existing) and treatment-acquired, poses a significant challenge to its effectiveness [66]. Several patients do not respond to trastuzumab initially, while others may develop resistance over time.

Aside from HER-2, the C-Met receptor tyrosine kinase is also frequently dysregulated in breast cancer. Lengyel et al. [81] evaluated the expression of the C-Met and HER-2 receptor in breast cancer as well as their lymph node metastases using both conventional immunohistochemistry and confocal immunofluorescence. The results showed that median disease-free survival in patients with C-Met overexpressing tumors was 8 months compared to 53 months when the expression was low (p=0.037; RR=3.0). Another study conducted by Liu et al. [82] investigated the efficacy of combinational therapy using trastuzumab (targeting HER-2) and crizotinib (targeting C-Met) in metastatic gastric cancer patients who had amplification of both HER-2 and C-Met genes. These patients were found to have a poor progression-free survival (PFS) prognosis. The rationale behind this combination therapy was based on the idea that targeting HER-2 and C-Met, both implicated in the progression and resistance of gastric cancer, may provide enhanced therapeutic benefits. Trastuzumab is an approved therapy for HER-2-positive gastric cancer, and crizotinib is a tyrosine kinase inhibitor that primarily targets C-Met but also has activity against other kinases. These results indicate that combination therapy has great potential as a treatment option for metastatic gastric cancer patients with concurrent HER-2 and C-Met amplification, even though there is room for improvement in terms of PFS.

C-Met and VEGF combination

Combination chemotherapy regimens that target both C-Met and vascular endothelial growth factor (VEGF) pathways have been explored in certain types of cancer [25,83]. The rationale behind this approach is to simultaneously inhibit multiple signaling pathways involved in tumor growth, angiogenesis (the formation of new blood vessels), and metastasis, potentially leading to improved treatment efficacy [25,84]. C-Met is a receptor tyrosine kinase involved in cell growth, survival, and migration, while VEGF is a key factor in promoting angiogenesis [85]. The inhibition of both signaling pathways may work synergistically to suppress tumor growth and inhibit the formation of new blood vessels that support tumor development [86].

Several drugs targeting C-Met and VEGF have been developed and evaluated in preclinical as well as clinical studies. For example, Cabozantinib, a tyrosine kinase inhibitor that targets both receptors showed great...
efficacy in certain cancers, such as advanced renal cell and hepatocellular carcinoma [62]. Another example is the combination of C-Met inhibitor crizotinib with anti-VEGF antibody bevacizumab [52]. This combination has been investigated in preclinical models and early-phase clinical trials for various malignancies, including non-small cell lung and colorectal cancer. Although the combination therapies targeting both pathways have shown promising results, their clinical effectiveness and safety profiles are still being evaluated through ongoing clinical trials. The availability and use of these therapies may vary depending on the specific cancer type and the stage of clinical development.

DISCUSSION

Several theories have been proposed to further enhance current understanding regarding the role of the HGF/C-Met pathway in ovarian carcinogenesis. In this study, the results showed that EOC cells maintain their proliferation through persistent activation of C-Met. This activation is attributed to the constant supply of HGF from other tissues through the bloodstream or increased expression of the ligand due to mutation. Additionally, the discovery of co-expression between C-Met and other growth factor receptors will elaborate on the possible combination of targeted chemotherapy to improve patient outcome and survival.

Ovarian cancer is characterized by its aggressive nature and high mortality rate, often due to late-stage diagnosis and limited treatment options. The dysregulation of HGF/C-Met signaling has been observed in ovarian cancer, promoting tumor growth, invasion, angiogenesis, the development of chemoresistance, and worsening poor patient outcomes [20,63]. One common mechanism of dysregulation in EOC is the upregulation or overexpression of the C-Met receptor through various genetic alterations, including gene amplification or mutation. The increased expression of C-Met leads to sustained activation of the pathway, promoting uncontrolled cell proliferation and survival in EOC cells [9,20]. Amplification of this gene has been recognized as a mechanism contributing to the development of resistance to EGFR-tyrosine kinase inhibitors (EGFR-TKI) [64].

Aberrant expression of HGF/C-Met signaling can also arise from the increased production of the HGF ligand. In EOC, the tumor cells or nearby stromal cells may produce an excessive quantity of HGF, either for their proliferation (autocrine) or to affect neighboring cells (paracrine) [56]. This heightened expression activates the C-Met receptor, resulting in continuous activation of downstream signaling pathways, and contributing to tumor growth as well as the spread of cancer cells to distant sites (metastasis). The majority of individuals diagnosed with EOC typically exhibit advanced disease characterized by peritoneal dissemination and the presence of ascites. A previous study found high HGF levels in ascitic fluids, known as soluble factors, which are responsible for metastasis [24]. Malignant ascites provide a protective environment for tumor cells. Many soluble factors and cytokines found in malignant ascites are associated with the invasion and migration abilities of cancer cells. A high concentration of HGF in ascitic patients results from self-expression by the tumor microenvironment.

Dysregulation of HGF/C-Met signaling in EOC may involve the activation of alternative signaling pathways. For example, in the absence of an HGF ligand, constitutive activation of C-Met potentially occurs through mutations or alterations in downstream signaling molecules [39]. These alterations can bypass the normal ligand-dependent regulation and lead to sustained C-Met activation, driving aberrant signaling in EOC cells. In this study, the mutation of the PI3K gene was found to be associated with the etiopathogenesis of both typical and non-typical endometriosis, which serves as an early step in ovarian carcinogenesis. Mutation of the Wnt/β-catenin pathway also leads to self-renewal of the cancer cells, chemoresistance, and metastasis in all subtypes of EOC. Increased expression of ligands, aberrant activation of receptors or intracellular mediators, disturbance of the β-catenin destruction complex, inhibition of β-catenin/E-cadherin association at the cell membrane, and abnormal enhancement of β-catenin/TCF transcriptional activity have been documented in epithelial ovarian cancer (EOC), particularly in the high-grade serous subtype [41].

In EOC, cross-activation of proteins with C-Met occurs when other proteins interact with the receptor, resulting in the activation of downstream signaling pathways related to proliferation and cell survival [14]. This cross-activation is mediated through various mechanisms, including protein-protein interactions, co-expression, and co-localization [89]. Several proteins implicated in this process are EGFR, p53, KRAS, and other TKR such as HER-2, and VEGFR [41]. The interplay between various proteins and signaling pathways plays a crucial role in this process. Moreover, phosphorylation and activation of one TKR can influence the activation of another, triggering alternative signaling cascades [90]. C-Met, in particular, shares downstream pathways related to cell proliferation and survival [8]. Cross-activation, either due to auto-signaling by cancer cells or mutation cascades occurs in cancer and its environment. This phenomenon is important in the development of potential drugs for EOC treatment.

Targeting the HGF/C-Met pathway in ovarian cancer is an attractive and promising therapeutic strategy. Several molecular therapies have been explored to inhibit C-Met activation and downstream signaling [21,23]. Small molecule inhibitors, such as crizotinib [23] [demonstrated great potential in preclinical and early
clinical results by effectively inhibiting C-Met activity and suppressing ovarian cancer growth. Combination therapies involving C-Met inhibitor and standard chemotherapeutic agents have also been investigated. For instance, the combination of a C-Met inhibitor with paclitaxel [64], a commonly used chemotherapy drug, produced synergistic effects in suppressing ovarian cancer cell proliferation and enhancing cell death.

Aside from small molecule inhibitors, monoclonal antibodies targeting C-Met have shown great potential in preclinical studies [65]. These antibodies can block the interaction between HGF and C-Met, thereby inhibiting downstream signaling cascades. Anti-C-Met antibodies exhibited anti-tumor effects, including reduced tumor growth and metastasis in ovarian cancer models. Several combinations of chemotherapy targeting C-Met have been documented. The rationale behind targeting two or more TKR is to address the issue of resistance that might arise from treatment with one single type.

C-Met inhibition can enhance chemosensitivity through several possible mechanisms. First, in tumors with acquired resistance to EGFR-TKI, C-Met acts as a bypass [41], compensating for the inhibited EGFR pathway. The inhibition suppresses the activation of this alternative signaling route, preventing the escape from EGFR-TKI-induced growth inhibition and promoting chemosensitivity [93]. Second, C-Met and EGFR signaling pathways share common downstream effectors, such as PI3K/AKT and MAPK/ERK. Inhibition of C-Met potentially leads to the downregulation of these shared pathways, which are crucial for cell survival and proliferation. This downregulation synergizes with EGFR-TKI therapy, further impairing cell survival mechanisms and enhancing chemosensitivity. Third, tumors acquire resistance to EGFR-TKIs through the emergence of secondary EGFR mutations [94]. C-Met inhibition has been shown to counteract the effects of these mutations and restore sensitivity to the therapy [95]. This is achieved by suppressing the compensatory signaling driven by mutant EGFR, rendering the tumor cells susceptible to EGFR-TKI-induced growth inhibition. The same mechanism applies to HER-2/C-Met and VEGFR/C-Met combination chemotherapy, where dual receptor blockade becomes the hallmark of treatment effectiveness.

There are certain challenges associated with the effectiveness of targeting HGF/C-Met signaling in ovarian cancer. Intra-tumoral heterogeneity, crosstalk with other signaling pathways, and acquired resistance mechanisms can limit the efficacy of therapeutic interventions. Therefore, a comprehensive understanding of the underlying molecular mechanisms, identification of predictive biomarkers, and development of combination therapies are essential for optimal targeting of HGF/C-Met signaling in ovarian cancer.

Advancements in precision medicine and the identification of patient subgroups with specific alterations in the HGF/C-Met pathway may enable more personalized treatment approaches. Molecular profiling of ovarian tumors to identify C-Met amplifications, mutations, or other alterations could help in selecting patients more likely to benefit from HGF/C-Met-targeted therapies.

CONCLUSIONS

HGF/C-Met signaling plays a crucial role in the tumorigenesis of ovarian carcinoma through a complex signaling cascade. Considering the intricacy of this pathway, there is a need to conduct further investigations on the downstream signaling pathways inhibited by deactivating C-Met. Recent studies showed that single-drug chemotherapy is more likely to lead to drug resistance. Consequently, investigating the combination of C-Met inhibitors with other TKI therapies can provide valuable insights into effective therapeutic options. Ongoing phase II/III clinical trials positioned these chemotherapeutic agents as promising second-choice therapy. C-Met receptors are also frequently co-expressed with other proteins, such as EGFR and KRAS, in cancer cells with overexpression. Therefore, the drug’s pharmacokinetics and pharmacodynamics, as well as its relationship with the C-Met receptor, must be accurately described.

DECLARATION

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