

## REVIEW

## Gelsolin: new insights into its roles on gastric cancer dissemination

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**Gastric cancer (GC) is one of the most prevalent cancers worldwide, with more than 700,000 cases of death annually. Histopathologically, GC can be classified into two main subtypes, the intestinal and diffuse type GC. These two subtypes differ not only in histological parameters, but also show distinct profiles of gene alterations. In this research highlight, we provide a summary of molecular mechanisms underlying tumor cell behavior in both the intestinal and diffuse type GC, and also highlight our recent findings on the roles of gelsolin, an actin-regulating protein, in GC dissemination. We recently found that gelsolin is differentially expressed in intestinal and diffuse type GC, and uncovered its involvement in the HGF/c-Met oncogenic pathway, which is a frequently activated signaling pathway in GC dissemination. Other roles of gelsolin in cancer development are also discussed, with a focus on its association with oncogenic pathways and gene alterations in cancer metastasis. Our work provides a potential link between gelsolin and pro-invasive pathways in GC, and hence suggests avenues for combating GC dissemination and metastasis with consideration of gelsolin status in tumor.**

**Keywords:** Gastric cancer; Gelsolin; E-cadherin; HGF/c-Met; PI3K; Cancer dissemination; metastasis

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### Introduction to Gastric Cancer

Gastric cancer (GC) remains one of the world's most common cancer types, and is more prevalent in men than in women across various countries <sup>[1]</sup>. Although its occurrence

has decreased over the years, it is still a leading cause of oncologic deaths, with over 700,000 deaths annually worldwide based on GLOBOCAN in 2012 <sup>[1, 2]</sup>. Significantly, *Helicobacter Pylori* (*H. pylori*) infection, which is a major risk factor of GC, accounts for 70 percent of

GC occurring in developing countries<sup>[3]</sup>. The mechanisms of *H. pylori*-induced gastric transformation include the evasion of host defense, enhanced inflammatory and immune responses, activation of signaling pathways such as nuclear factor-kappa B (NF- $\kappa$ B) and Wnt/ $\beta$ -catenin, and perturbation of metal ions<sup>[4]</sup>. Following sustained infection, non-atrophic gastritis first develops into multifocal atrophic gastritis without metaplasia, then into metaplasia and dysplasia<sup>[5, 6]</sup>. Moreover, GC also has a direct correlation with the consumption of tobacco, alcohol and a diet rich in starch but poor in protein quality, fruits and vegetables<sup>[7]</sup>.

Most GC are adenocarcinomas arising from gastric epithelium while other less common types include sarcoma, lymphoma and carcinoids. Common sites of gastric adenocarcinomas include gastric cardia, antrum, and body of the stomach<sup>[8]</sup>. According to Lauren classification, there are two main histologic subtypes of adenocarcinoma: intestinal and diffuse type<sup>[9]</sup>. The intestinal type GC displays well-differentiated tubular or glandular structure, and characteristically forms either an ulcerated tumor or an exophytic mass. In contrast, the diffuse type GC presents undifferentiated or poorly differentiated glandular structures with infiltration of neoplastic cells into mucosa or gastric wall.

In general, GC development is associated with multiple genetic alterations<sup>[10, 11]</sup> (Table 1), which contribute to genomic instability and hence promote tumorigenesis. These alterations could be classified into mutations in tumor suppressor genes (Adenomatous polyposis coli (APC) and p53<sup>[12, 13]</sup>), oncogenes (c-Myc and K-ras), growth factor signaling (Hepatocyte Growth Factor (HGF)<sup>[14, 15]</sup> and Epidermal Growth Factor (EGF)<sup>[16]</sup>, cell-cycle regulators (cyclin E<sup>[17]</sup>), cell adhesion and metastatic-related genes (E-cadherin<sup>[18]</sup> for instance), DNA-repair genes and epigenetic factors<sup>[19, 20]</sup>. Among these mutations, several mutations such as p53 and Hepatocyte growth factor/hepatocyte growth factor receptor (HGF/c-MET)<sup>[11]</sup> can be commonly found in both intestinal type and diffuse type GC. On the other hand, the two subtypes of GC also exhibit distinct mutations, giving rise to different genetic backgrounds. It is observed that intestinal type GC generally displays molecular signatures represented by enhanced cellular growth whereas in the diffuse type GC, gene clusters of cellular and extracellular matrix (ECM), adhesion, interaction and migration, immune response and several metabolism pathways are observed to be more up-regulated<sup>[21, 22]</sup>. In particular, diffuse type GC has shown to be highly associated with the loss of E-cadherin function, arising from CDH1 gene mutations<sup>[20]</sup>, while the intestinal type GC is associated with other genetic abnormalities including mutations in APC and  $\beta$ -catenin (CTNNB1)<sup>[21, 23]</sup>, a protein

that binds to both E-cadherin and APC protein.

Apart from the above mentioned genes, our laboratory has recently discovered that gelsolin, an important actin regulator, is also differentially expressed in these two subtypes of GC<sup>[24]</sup>.

### Gelsolin in cancer

Gelsolin, and its other family members, are important actin-regulating proteins which regulate actin dynamics and are essential in many biological events such as motility, adhesion, secretion, and cell death<sup>[25]</sup>. The conventional role of gelsolin involves the regulation of actin filament turnover via the severing and capping processes, which are further regulated by several secondary messengers such as calcium and phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>)<sup>[25]</sup>. In addition, there are accumulating evidences suggesting gelsolin's involvement in pathological conditions including cancer.

In cancer, the expression levels of gelsolin appear to be affected by the types of cancer and cancer cell behavior. Gelsolin has been shown to be down-regulated in several types of cancer, including breast<sup>[26]</sup>, prostate<sup>[27]</sup>, ovarian<sup>[28]</sup>, and bladder cancers<sup>[29]</sup>. In contrast, parallel studies reported that increased gelsolin levels correlate with aggressive tumor behavior in some types of cancer such as non-small cell lung cancer<sup>[30, 31]</sup>, oral cancer<sup>[32]</sup>, cervical cancer<sup>[33]</sup>, pancreatic cancer<sup>[34]</sup>, urothelial tumor<sup>[35]</sup>, hepatocellular carcinoma<sup>[36]</sup>, and renal cell carcinoma<sup>[37]</sup>. In these studies, expression of gelsolin frequently correlates with poor prognosis such as reduced disease-free survival, and aggressive tumor behavior like metastasis and lymphatic invasion. One hypothesis that can explain the discrepancy of gelsolin expression in cancers is that gelsolin is down-regulated at early stages of tumorigenesis, but it is re-expressed as the tumor progresses and its expression contributes to the aggressiveness of cancer. This hypothesis is supported from studies in urothelial carcinoma and oral cancer<sup>[32, 35]</sup>. However, whether this hypothesis applies to specific types of cancer or is a general phenomenon in cancers still needs to be tested. These contradicting observations of gelsolin expression suggest complex regulation and roles of gelsolin in cancer progression, and the need to investigate its functions in tumors with different genetic backgrounds.

A number of *in vitro* and *in vivo* studies have demonstrated that gelsolin is a critical factor in cancer cell migration and invasion, which are crucial steps in metastasis. Knockdown of gelsolin counteracts the invasive capacity of a panel of cancer cell lines including breast<sup>[38]</sup>, cervical<sup>[38]</sup>, pancreatic<sup>[34]</sup>, colorectal<sup>[39, 40]</sup>, melanoma<sup>[39]</sup>, and

**Table 1. A brief summary of genetic alterations in diffuse type and intestinal type GC**

	GENETIC ALTERATIONS	INCIDENCE			
		DIFFUSE TYPE	INTESTINALTYPE		
		(%)	(%)		
<b>Tumor Suppressor Genes</b>	Adenomatous polyposis coli (APC)	Mutation <sup>[85]</sup>	13.2	33.3	
	Deleted in colorectal cancer (DCC)	LOH <sup>[86]</sup>	43.8	37.5	
	Runt-related transcription factor 3 (RUNX3)	Reduced/Loss of Expression <sup>[87]</sup>	17.4	80.8	
	p53	Mutation <sup>[88]</sup>	4.2 <sup>^</sup>	41.0 <sup>^</sup>	
		Overexpression <sup>[89]</sup>	46.4	44.0	
<b>Oncogene</b>	c-Myc	Overexpression <sup>[90]</sup>	19.0	9.1	
	K-ras	Mutation <sup>[91]</sup>	9.1	8.5	
<b>Growth Factors &amp; Receptors</b>	Hepatocyte Growth Factor Receptor (HGFR/c-MET)	Overexpression <sup>[92]</sup>	61.4	33.8	
	Epidermal Growth Factor Receptor 2 (HER2/c-erbB-2)	Gene amplification <sup>[92]</sup>	1.8	23.0	
		Overexpression <sup>[92]</sup>	3.5	26.8	
	Fibroblast Growth Factor Receptor2 (FGFR2/K-sam)	Overexpression <sup>[93, 94]</sup>	4.5 - 37.0	2.1 - 19.0	
	Vascular endothelial growth factor (VEGF)	Overexpression <sup>[95]</sup>	42.9	68.9	
<b>Cell Cycle &amp; Apoptotic related proteins</b>	Cyclin D1	Overexpression <sup>[96]</sup>	35.2	52.6	
	Cyclin E2	Overexpression <sup>[96]</sup>	8.8	13.1	
	B-cell lymphoma 2 (Bcl-2)	LOH <sup>[97]</sup>	0	36.0	
		Overexpression <sup>[98-100]</sup>	11-25	15.7-71.2	
<b>Cellular Matrix related proteins</b>		Aberrant Expression <sup>*[101]</sup>	72.5	33.3	
	E-cadherin (CDH1)		38.4	27.5	
		LOH, Mutation, Epigenetic Alteration <sup>[102]</sup>	(52.6)	(30.2)	
	β-catenin (CTNNB1)	Mutation <sup>[23, 103]</sup>	0-38	23-26.9	
		Aberrant Expression <sup>*[101]</sup>	70.0	31.5	
		Matrix metalloproteinase 1 (MMP-1)	Overexpression <sup>[104]</sup>	55.6	82.9
		Matrix metalloproteinase 9 (MMP-9)	Overexpression <sup>[104]</sup>	63.0	87.2
<b>Tumor Associated Proteases</b>	Urokinase-type plasminogen activator (uPA)	Overexpression <sup>[105]</sup>	33.3	65.3	
	Urokinase-type plasminogen activator Receptor (uPAR)	Overexpression <sup>[105, 106]</sup>	50.0 - 60.0	52.3 - 75.5	

<sup>^</sup> Include Early and Advanced Gastric Cancer. ( ) Familial Cases Gastric Cancer- Hereditary diffuse gastric cancer (HDGC) and Familial intestinal gastric cancer (FIGC). \*Aberrant refers to loss of membranous expression, including those with absent, heterogeneous or cytoplasmic staining patterns

hepatocellular carcinoma cells <sup>[36]</sup>, while overexpression of gelsolin enhances the migration and invasiveness of many cancer cells <sup>[36, 39, 40]</sup>. Interestingly, we previously found that gelsolin is up-regulated in metastatic variants of HCT116 colorectal cancer cells <sup>[40]</sup>, and the distribution of gelsolin

shows a peripheral localization with F-actin in an invasive variant of LS180 colon adenocarcinoma cells in a previous report <sup>[41]</sup>, suggesting that the localization and proper function of gelsolin might be important for its invasion-related effects. Due to its pro-invasive roles,

gelsolin can be suppressed by tumor suppressors to counteract metastasis. Such down-regulation of gelsolin has been demonstrated in Nm23-H1-mediated metastatic suppression in breast cancer [42]. Controversially, there is a study showing that gelsolin could inhibit invasion of bladder cancer cells with activating transcription factor 3 (ATF3) knockdown background [43]. The reason for such discrepancy is unknown at the moment, but it might be due to different genetic backgrounds of different cancer types.

### Gelsolin expression in GC subtypes

In line with the well-established pro-migratory and pro-invasive roles of gelsolin, our recent study reported a crucial role of gelsolin in promoting GC dissemination. Compared to intestinal type GC, we have shown that gelsolin is up-regulated in the diffuse type GC, which is characterized by cancer cell infiltration [24]. Gelsolin expression is also higher in lymph node metastases compared to their primary intestinal tumors. Our observation suggests that gelsolin is up-regulated in a subset of GC, and contributes to the aggressiveness of cancer, in terms of dissemination and invasion.

### Gelsolin and E-cadherin in GC

E-cadherin is a classical Type I cadherin protein and is essential in the maintenance and modulation of intracellular cadherin-mediated cell-cell adhesion, signaling and cytoskeleton organization [44]. E-cadherin has been shown to possess tumor suppressive properties where the repression of E-cadherin expression serves as a key event in initiating epithelial-mesenchymal transition (EMT) [45]. This subsequently results in the disruption of the cellular adhesion dynamics and acquisition of a phenotype with increased migratory and invasive capabilities arising from a wide range of transcriptional and functional changes [46]. In GC, loss of E-cadherin function has been reported especially in diffuse-type GC, and the loss of E-cadherin has been associated with poorer differentiation, increased motility, invasion and metastatic potential [47-49]. This loss of E-cadherin can be attributed to epigenetic and genetic modifications including promoter methylation of CDH1 gene [50], loss of heterozygosity (LOH) [51], inactivating germline and somatic mutations [18, 52]. Besides genetic alterations, alternative mechanisms can inhibit E-cadherin activity in cancer by reducing E-cadherin expression through processes like increased endocytosis [53], proteolytic processing of E-cadherin [54], and activation of its transcriptional repressors [55]. As summarized (Table 1), the alteration of the E-cadherin gene is observed in about one third of GC, while the aberrant function or expression of E-cadherin have been

reported in the majority of diffuse type GC. Therefore, non-genetic regulation could play an important role in E-cadherin expression and function in GC.

In our study, we identified an alternative pathway leading to E-cadherin repression, which involves gelsolin and its downstream pathways. We reported that gelsolin expression correlated negatively with wild-type E-cadherin in three cohorts of GC patients, and was shown to suppress the expression of E-cadherin at both mRNA and protein levels in a panel of human GC cell lines. Concurrently, several E-cadherin transcriptional repressors, including Snail, Twist1, and Zeb2, are up-regulated by gelsolin, which are consistent with the decreased levels of E-cadherin observed. Changes in mRNA levels of E-cadherin and its repressors were also observed in our study upon the knockdown of gelsolin in GC.

Our observations on transcriptional changes after altering gelsolin levels suggest that gelsolin might have the ability to regulate mRNA changes and possibly gene transcription. Although it has not been reported to have DNA binding sites, gelsolin has been observed to be a transcriptional co-activator and participate in the regulation of gene expression [56, 57]. Gelsolin was shown to interact with androgen receptor (AR), which leads to the nuclear translocation and enhanced transcriptional activity of AR [56]. Likewise, gelsolin has also been shown to interact with thyroid hormone receptor  $\beta$  and Hypoxia-inducible-factor-1 $\alpha$  (HIF-1 $\alpha$ ), which might lead to the regulation of their transcriptional activities [57, 58]. More recently, gelsolin was suggested to be a crucial factor in mediating the assembly and/or stability of estrogen receptor  $\beta$  complexes in the nucleus [59]. These findings suggest gelsolin act as a transcriptional co-activator of several transcription factors. In support of this, there has been mounting evidences showing that modulation of gelsolin levels leads to alterations in the mRNA levels of several genes, such as urokinase-type plasminogen activator (uPA) [40]. Although changes in mRNA levels of E-cadherin and several genes were observed after silencing gelsolin in GC, the mechanisms of how gelsolin regulates these changes are still unknown at the moment.

Furthermore, as E-cadherin is a master regulator of EMT as mentioned previously, our findings also suggest that gelsolin might play a role in EMT in GC. The influence of gelsolin on EMT has been suggested in other types of cancer. In cervical cancer, knockdown of gelsolin up-regulated epithelial markers such as E-cadherin while down-regulating mesenchymal markers such as vimentin, and ECM-degrading enzymes including matrix metalloproteinase (MMP)-2 and -9

[33]. The suppressive effect of gelsolin on E-cadherin expression has also been observed in cardiomyocytes [60]. More recently, gelsolin is suggested to play a role in Transforming Growth Factor (TGF)- $\beta$ 1 induced EMT changes [61], where gelsolin is found to be epigenetically up-regulated upon TGF- $\beta$ 1 treatment in breast cancer cells, together with decreased epithelial marker E-cadherin and increased mesenchymal markers including N-cadherin and vimentin. Overexpression of gelsolin effectively increases vimentin while knockdown of gelsolin reverses this effect.

Together with these evidences, our study points out the possible involvement of gelsolin in EMT in GC, which could eventually contribute to metastasis. Moreover, as mentioned above, there are evidences suggesting that gelsolin could act as a transcriptional co-factor of AR and hence, it is possible that gelsolin may also act in similar ways to be co-activators of transcription factors regulating E-cadherin and EMT-related gene expression. Future studies may be conducted to address the potential roles of gelsolin as a

transcriptional regulator to promote GC dissemination.

### Gelsolin and HGF/c-MET pathway signaling in GC

The HGF/c-MET pathway has been shown to be essential in facilitating cancer progression in GC as well as other types of cancer, whereby the aberrant activation of c-MET can promote tumor growth and increase both invasiveness and metastatic potential through several downstream signaling pathways. These changes include activation of Signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase/ extracellular signal-regulated kinase (MAPK/ERK), phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/Akt), and HIF-1 $\alpha$ -induced activity [62-65]. Furthermore, HGF signaling was also shown to modulate functional E-cadherin, a master regulator of EMT, possibly contributing to the increased invasive capabilities of GC [66]. In GC, hyperactivation of HGF/c-MET can be attributed to the genetic amplification of the proto-oncogene c-MET and the increase in both autocrine and paracrine secretion of HGF

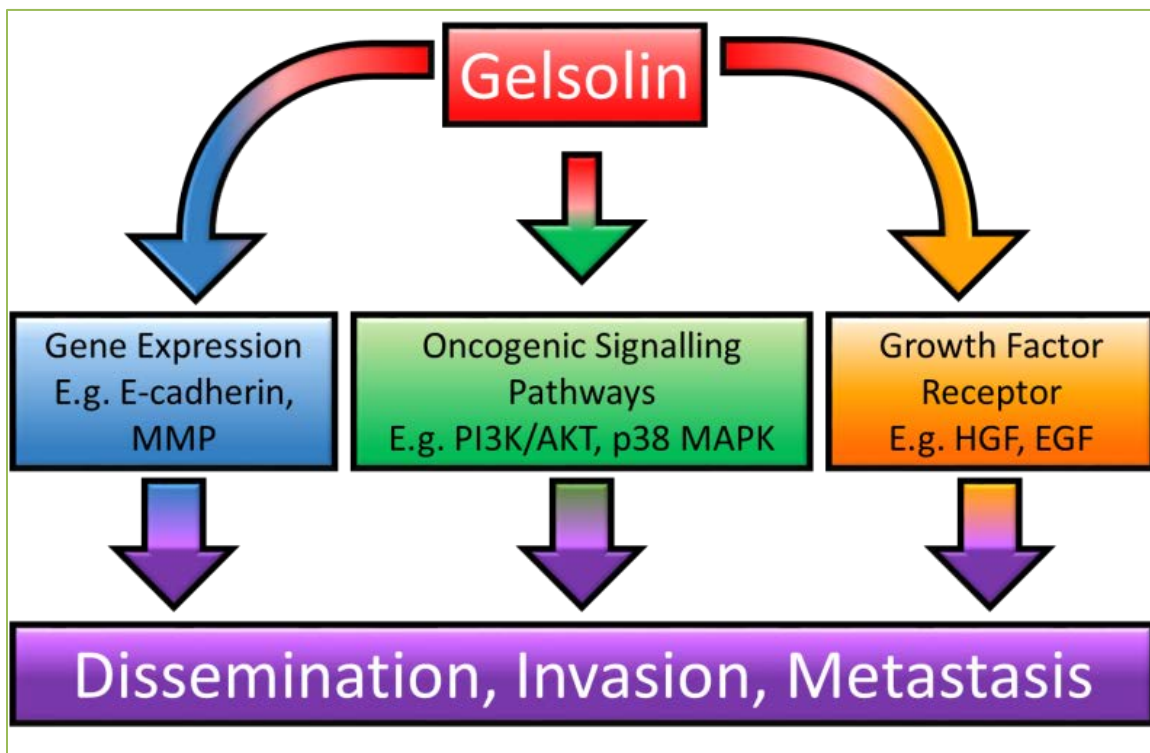


Figure 1. A summary of gelsolin in cancer invasion and metastasis.

by stroma cells in tumor microenvironment [67-69]. Downstream activation of these signaling networks can result in increases in cellular proliferation and survival, motility and scattering, matrix degradation and infiltration of tissues, and stimulation of angiogenesis [62, 63, 70], thereby contributing to HGF-mediated tumor progression and EMT.

Our recent study has identified that gelsolin modulates HGF/c-MET activation and its downstream effects. We have shown that HGF treatment increases expression levels of gelsolin, and that gelsolin modulates the HGF-induced scattering of GC cells, PI3K/Akt pathway activation, and subsequent gene expression. Our study has revealed a novel

role of gelsolin in mediating HGF/c-MET-induced scattering. In line with our observations, villin, a gelsolin family member, has also been suggested to modulate HGF-induced scattering<sup>[71]</sup>. Previous reports have also identified the roles of gelsolin in modulating cellular responses and downstream effects of other secreted growth factors, including neuroendocrine factor in neurotensin-induced invasion of prostate cancer cell and EGF in EGF-induced motility and invasion<sup>[72-74]</sup>. Taken together, our observations and other studies highlight the role of gelsolin in invasion triggered by growth factors. It might therefore be important to consider gelsolin status in HGF/c-MET targeted therapy and probably other therapies targeting growth factors which could be influenced by gelsolin.

### Gelsolin and PI3K/Akt pathway in GC

PI3K/Akt pathway, which can be activated downstream of HGF/c-MET and other growth receptors, is critical for tumorigenesis, survival and metastasis in GC<sup>[75]</sup> where the increase in PI3K/Akt activity enhances metastasis and correlates with poorer prognosis of GC patients. The increased PI3K/Akt activity can be attributed to the amplification of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA)<sup>[76, 77]</sup>, elevated level of Akt/p-Akt<sup>[78, 79]</sup>, as well as genetic and epigenetic alterations of phosphatase and tensin homolog (PTEN)<sup>[80]</sup>. Targeted inhibition of PI3K/Akt, such as through small molecules, has been shown to result in the inhibition of proliferative and metastatic capabilities of GC<sup>[81]</sup>.

Our study also reveals the involvement of gelsolin in activating the PI3K/Akt pathway to enhance HGF-induced E-cadherin repression and up-regulation of its repressors. Using proximity ligation assay, we have uncovered that gelsolin could interact with PI3K, which might contribute to its effect on PI3K/Akt activation. Consistent with our findings, gelsolin has been found to be a binding partner of PI3K and other members of podosome signaling complex<sup>[82]</sup>. The interaction between gelsolin and PI3K could lead to enhanced activity of PI3K<sup>[83]</sup>, highlighting the role of gelsolin in recruitment and activation of signaling molecules. Furthermore, gelsolin-mediated invasion is dependent on Ras-PI3K-Rac signaling<sup>[84]</sup>. Taking together, the above mentioned studies suggest a potential involvement of gelsolin in PI3K oncogenic signaling pathway and its importance in gastric carcinogenesis and metastasis.

### Conclusion

In conclusion, GC is characterized by multiple genetic alterations, including mutations and activation of oncogenic

pathways like HGF/c-MET and PI3K/Akt. One of the consequences of those alterations is enhanced or acquired capacity of GC cells to invade and metastasize. Gelsolin has been shown to regulate invasion and metastasis in several types of cancer, which involves the alteration of signaling pathways and gene expression (Figure 1). Recent findings from our laboratory have shown that gelsolin modulates the HGF/c-MET response and PI3K/Akt activation, leading to suppression of E-cadherin, which in turn promotes the dissemination and metastasis of GC. Our observation provides a potential link between gelsolin and pro-invasive pathways, and hence a more in-depth look into gelsolin expression status in gastric cancer might provide another avenue for combating GC dissemination and metastasis.

### Conflicting interests

The authors have declared that no conflict of interests exist.

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### Author contributions

All authors (S.D., M.S.O., Z.X.N., T.J., J.B.Y.S., A.P.K., and C.T.Y.) contributed to the writing of the paper and revision of the manuscript.

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