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Contents

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Contents

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Contents

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WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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META-ANALYSIS

Success rate of current human-derived gastric cancer organoids establishment and influencing factors: A systematic review and meta-analysis

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Abstract

BACKGROUND

Human-derived gastric cancer organoids (GCOs) are widely used in gastric cancer research; however, the culture success rate is generally low.

AIM

To explore the potential influencing factors, and the literature on successful culture rates of GCOs was reviewed using meta-analysis.

METHODS

PubMed, Web of Science, and EMBASE were searched for studies. Two trained researchers selected the studies and extracted data. STATA 17.0 software was used for meta-analysis of the incidence of each outcome event. The adjusted Methodological Index for Non-Randomized Studies scale was used to assess the quality of the included studies. Funnel plots and Egger's test were used to detect publication bias. Subgroup analyses were conducted for sex, tissue source, histological classification, and the pathological tumor-node-metastasis (pTNM) cancer staging system.

RESULTS

Eight studies with a pooled success rate of 66.6% were included. GCOs derived from women and men had success rates of 67% and 46.7%, respectively. GCOs from surgery or biopsy/endoscopic submucosal dissection showed success rates of 70.9% and 53.7%, respectively. GCOs of poorly-differentiated, moderately-differentiated and signet-ring cell cancer showed success rates of 64.6%, 31%, and 32.7%, respectively. GCOs with pTNM stages I-II and III-IV showed success rates of 38.3% and 65.2%, respectively. Y-27632 and non-Y-27632 use showed success

rates of 58.2% and 70%, respectively. GCOs generated with collagenase were more successful than those constructed with Liberase TH and TrypLE (72.1% *vs* 71%, respectively). EDTA digestion showed a 50% lower success rate than other methods ($P = 0.04$).

CONCLUSION

GCO establishment rate is low and varies by sex, tissue source, histological type, and pTNM stage. Omitting Y-27632, and using Liberase TH, TrypLE, or collagenase yields greater success than EDTA.

Key Words: Gastric cancer organoids; Human-derived organoids; Gastric cancer; Cell lines; *In vitro* research models

Core Tip: This study systematically reviewed the success rate of establishing human-derived gastric cancer organoids (GCOs), highlighting the relatively low overall success rate that is influenced by factors such as sex, tissue source, histological type, and pathological tumor-node-metastasis cancer stage. Our meta-analysis revealed that omitting the Rho Kinase inhibitor Y-27632 and using certain digestive enzymes, such as collagenase, enhanced culture success. These findings suggest potential avenues for improving GCO culture techniques that are crucial for advancing gastric cancer research and personalized medicine.

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INTRODUCTION

Gastric cancer (GC) is a malignant tumor prevalent worldwide with high mortality and morbidity and poses a serious threat to human health[1]. Due to a poor prognosis, surgery is currently the only possible curative treatment for GC[2]. However, surgery alone is not sufficient for GC, and it is often clinically necessary to combine it with preoperative chemotherapy that has become a routine treatment option for improving long-term survival[3]. Patients with advanced stage GC require systemic chemotherapy, targeted therapy, and immunotherapy. Despite the development of new treatment options, the lack of suitable *in vitro* research models and difficulties in conducting clinical trials hinder progress in personalized and precise treatments[4].

Currently, the primary obstacles in cancer research are the lack of suitable tools for *in vitro* research models and the difficulty of starting clinical trials directly with patients to achieve personalized and precise treatment. Traditional disease models include animal and cellular formats that can be divided into *in vivo* and *in vitro* models[5]. However, differences in the species and structure of objects often prevent animal models from accurately simulating the real psychophysiological processes in humans. *In vitro* models are based on cell culture technology and bring with them the advantages of homology, replication, monoculture, and unlimited proliferation[6]. Despite these advantages, the number of GC cell lines available for study is insufficient to comprehensively cover the vast spectrum of various cancers. Moreover, most established tumor cell lines are derived from metastatic or rapidly progressing tumors; therefore, primary or slowly progressing tumors cannot be identified and employed in research. Additionally, cell lines eventually undergo senescence after a finite number of cell divisions and are viable for less than 1 year. Furthermore, primary cell lines cannot be cultured a long period of time[7] and a more suitable tool is needed to establish an *in vitro* research model.

Organoids consist of a cluster of cells derived from stem cells that have self-organizing and self-renewal capabilities that can better preserve the functional and histological properties of the original organ. Organoids have been cultured from various organs, including the brain, retina, kidney, liver, intestine, and stomach[8-12]. There are two main sources of organoids: Organ-restricted adult stem cells (ORISC) and pluripotent stem cells (PSCs)[13,14] that include induced PSCs and embryonic stem cells. PSC-derived organoids rely on the artificial induction of interactions between important signaling pathways during development *in vivo*, whereas ORISC-derived organoids retain the inherent genetic information of the original tissue[13]. Unlike PSC-derived organoids, ORISCs do not contain cellular microenvironmental components but retain the properties of the source tissue to a greater extent[15]. Three-dimensional (3D) structural GC spheroids were first constructed in 2013[16]. In 2015, the first tumor organoid bank was established[17]. Researchers have gradually established organoid banks for the treatment of various tumors. Bartfeld *et al*[18] were the first to report that human-derived GC organoids (GCOs) could be grown in the laboratory, and this marked a new area in GC research.

GCOs are widely used in basic research on GC genomic and transcriptomic analyses, drug screening, xenografts, and physiology. The successful construction of GCOs is undoubtedly the basis for promoting organoid research and its applications. However, the current success rate of GCO culture is generally low. This systematic review analyzes the current literature on the rate of successful culture of GCOs using meta-analysis and explores the factors impacting this

issue.

MATERIALS AND METHODS

Data sources and searches

The PubMed, Web of Science, and EMBASE databases were searched from the dates of their inception until September 29, 2023 to locate candidate studies. The following terms were combined to generate search keywords: ("organoid" OR "gastroid" OR "spheroid") AND ("gastric cancer" OR "gastric tumor" OR "gastric carcinoma") AND ("patient" OR "human" OR "human-being").

Literature screening and data extraction

Two reliably trained objective researchers with expertise in the subject matter of the meta-analysis independently selected the papers and extracted the data based on the inclusion/exclusion criteria, and the selections were cross-checked. Disagreements were resolved by referring the issue to a third experienced researcher. Data were extracted according to the pre-established full-text data extraction checklist that included: (1) Basic characteristics of studies, such as authors and year of publication; (2) Patient characteristics, such as sex, tissue source, histologic classification, and pathological tumor-node-metastasis (pTNM) cancer stage classification[19]; and (3) Successful and unsuccessful establishment of GCO culture, GCO morphology, passage number, culture medium change time, and growth factors employed.

Inclusion and exclusion criteria

Inclusion: (1) Literature on the successful establishment of human-derived organoids. The criteria for successful organoid construction should include at least one of the following conditions. The constructed organoids should possess unique cellular morphology and tissue structure. Organoids should demonstrate sustained proliferation and growth, leading to the formation of observable organ-like structures. The constructed organoids should express distinctive differentiation markers required for specific cell types and exhibit a functionality similar to that of the original tissue. The constructed organoid should express specific genes and signaling pathways relevant to the gene expression pattern and signal transduction of the original tissue. The constructed organoid should be capable of responding to stimuli and exhibiting a responsiveness similar to that of the original tissue; (2) The disease type should be GC; and (3) Data on the organoid establishment success rate should be available.

Exclusion: (1) Animal experiments; (2) Repeated literature; (3) Unavailable data; (4) Incomplete culture data; and (5) Review, conference, book or document.

Quality assessment and statistical analysis

There is currently no accepted tool for evaluating the quality of cellular studies, as the included studies only calculate the pooled culture success with no control group. The adjusted Methodological Index for Non-Randomized Studies (MINORS) scale was applied to assess the quality of the included literature that comprised eight entries with a total of 16 points[20]. Funnel plots and Egger's test were used to detect publication bias. Statistical significance was set at $P < 0.05$ (two-sided). STATA 17.0 software was used for the meta-analysis of the incidence of each outcome event. Heterogeneity among studies was estimated using the χ^2 test and I^2 statistics. If P was < 0.1 and I^2 was > 50%, heterogeneity was deemed to be present among the included studies, and the random effects model was used for combined analysis. Otherwise, a fixed effects model was used. An additional subgroup analysis according to sex, tissue source, differentiation type, pTNM stage, growth factors employed, and digestive enzymes used was conducted to probe the influencing factors.

RESULTS

Characteristics of the retrieved literature

Based on the search strategy described above, 1006 studies were retrieved from online databases. After removing duplicate and irrelevant records, 699 articles were retained. Subsequently, 185 animal studies, 108 reviews, 285 conferences, and 46 books or documents were excluded from this dataset, while 57 of the remaining 75 articles were excluded due to unavailable data. Eighteen studies reported 302 cases of successful establishment of GCOs that were systematically analyzed[21-38]. Among them, 10 studies only reported success establishment which was lack of total establishment. Eight studies that reported both success and failure in establishing 265 GCOs were included for meta-analysis and five of these eight studies also reported detailed clinical information about the cases. The pooled success rate was calculated among these 8 studies. The flow chart of the retrieved literature is shown in Figure 1.

Characteristics of GCOs

Currently, GCOs are primarily obtained *via* endoscopic biopsy or surgery. Different histological types of GCOs, such as intestinal, diffuse, mixed, neuroendocrine carcinomas (NEC) and signet-ring cell cancers (SRCC), have been established. Six researchers also focused on the molecular subtypes; microsatellite instability (MSI), chromosomal instability, genomically stable, microsatellite stable (MSS), human epidermal growth factor (EGF) receptor 2, and Epstein-Barr virus subtypes of GCOs were successfully established. GCOs can be successfully constructed for all types of differentiation and

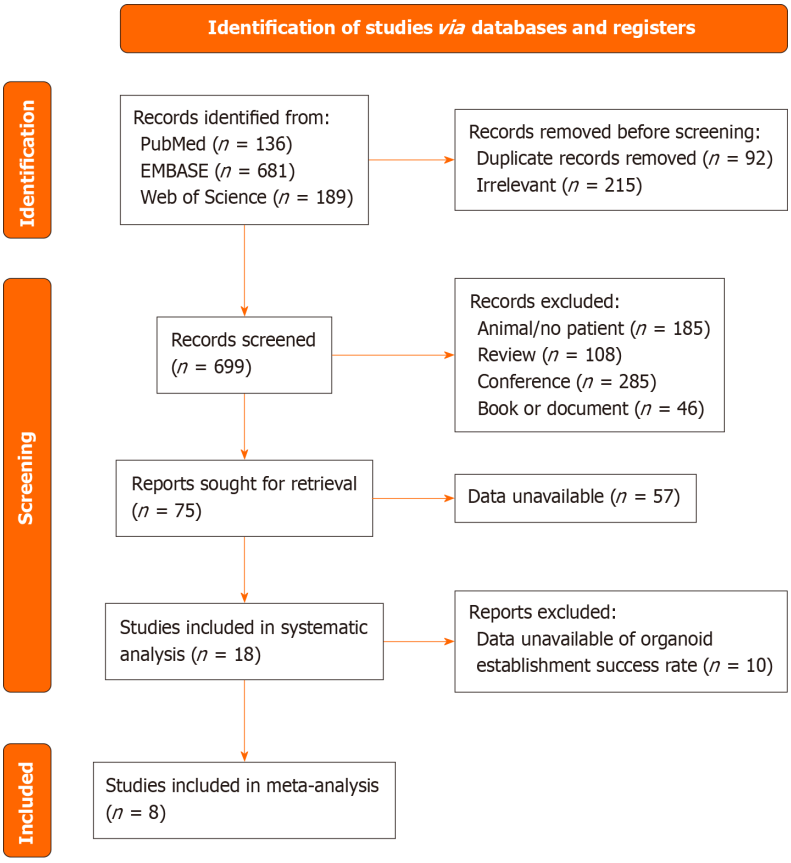


Figure 1 A flow diagram illustrating the screening process for studies eligible for meta-analysis. Initially, 1006 records were identified, with 699 records screened after removing duplicates and irrelevant entries. After further exclusions based on criteria such as relevance and data availability, 75 reports were considered for retrieval. Ultimately, 18 studies were included in the systematic review, with 8 of these meeting the criteria for inclusion in the meta-analysis.

pTNM cancer stages. The clinical characteristics of GCOs are shown in Table 1. The GCOs exhibited different morphologies in the wells. Most had a cystic structure, while glandular, solid, and grape-like structures were also observed in several studies. For GCOs culture, digestion was necessary, the culture medium had to be changed every 2-4 d, and the GCO had to be passaged every 2-14 d. The culture characteristics are presented in Table 2. Almost all studies included the following growth factors in the medium: Wnt3a, R-spondin-1, EGF, fibroblast growth factor (FGF)10, A83-01, and Noggin that were applied to the medium to achieve a high success rate. Table 3 shows the growth factors used in the GCOs culture. A total of 177 GCOs were successfully cultured from 265 samples. The pooled successful culture rate was 66.6% [95% confidence interval (CI): 0.468-0.840, I^2 = 88.77%], and a random-effects model was used (Figure 2).

Quality assessment

All studies included in the meta-analysis were of moderate-to-high quality as determined by using the adjusted MINORS scale. The majority of the studies reported long-term culture and passage of GCOs for at least 90 d, scoring a 2 in the "follow-up period" category. However, four studies received a score of 1 in the "baseline equivalence" category due to contamination leading to confounding factors. Table 4 shows the scoring criteria and quality assessments based on the adjusted MINORS tool.

Publication bias

Egger's test (P = 0.029) indicated the existence of a publication bias. The funnel plot is shown in Figure 3.

Subgroup analysis

Five studies that provided detailed clinical information on all lines of successful and failed construction of GCOs were further analyzed by different subgroups[21-27,37] (Table 5).

Sex

The combined construct success rate of 14 GCOs derived from women in four studies was 67.0% (95%CI: 31.1-95.7) and this was higher than that of GCOs derived from men (46.7%, 95%CI: 31.5-62.2) (Figure 4A).

Tissue source

Sixteen biopsy-derived GCOs from three studies were established successfully with a pooled success rate of 53.7% (95%CI: 27.1-79.5). Twenty-eight surgery- and endoscopic submucosal dissection (ESD)-derived GCOs from four studies

Table 1 The clinical characteristic of gastric cancer organoids

Ref.	Location	Molecular subtype	Histologic classification	Differentiation	pTNM stage	Source
Yan *et al*[21]	Body, fundus, cardia, antrum	EBV, MSI, Mixed	Intestinal, diffuse, mixed mucinous, mixed	Moderate, poor	IA-IIIC	Surgery
Kawasaki *et al*[22]	NM	NM	NET, NEC	NM	NM	Surgery, biopsy
Nanki *et al*[23]	GEJ, corpus, antrum	MSI, CIN, GS, MSS	Intestinal, diffuse, mixed	NM	IA-IV	Surgery, biopsy, ascites puncture
Steele *et al*[24]	Fundus	NM	Intestinal, diffuse, signet-ring cell	Poorly differentiated	NM	Surgery
Li *et al*[25]	Antrum, cardia, body	NM	Intestinal, diffuse, mixed	Poor, moderate, high	IA-IV	Surgery
Zou *et al*[26]	NM	NM	Intestinal, mixed, SRCC	Poor, moderate	IIB-IVA	Surgery, biopsy
Li *et al*[27]	Antrum, corpus, cardia	NM	NM	Poor, signet-ring cell, middle	NM	Biopsy
Togasaki *et al*[28]	NM	GS, CIN	Diffuse	Poor, sig, muc	IIA-IV	Surgery, ascites, biopsy, autopsy
Xiao *et al*[29]	NM	MSI, HER2, EBV	Adenocarcinoma	Moderate to poor differentiation	III	Surgery
Seidlitz *et al*[30]	NM	NM	Intestinal, diffuse, mixed	NM	IIA-IV	Surgery
Gao *et al*[31]	NM	NM	Adenocarcinoma	NM	NM	Surgery, biopsy
Bartfeld *et al*[32]	Corpus	NM	NM	NM	NM	Surgery
Wang *et al*[33]	Cardia, corpus	NM	SCRR	Poorly differentiated	IIB-III	Biopsy
Kumar *et al*[34]	Antrum, distal, corpus, pylorus, cardia, proximal, peritoneum	GS, MSI, CIN	Intestinal, diffuse, mixed	NM	I-IV	Biopsy
Harada *et al*[35]	NM	NM	Por, tub1, tub2, muc	Differentiated, undifferentiated	IA-IIIC	Surgery
Ukai *et al*[36]	NM	NM	Intestinal, diffuse, tub1, tub2, muc, pap	Por	IA-IIIC	Surgery
Tong *et al*[37]	NM	MSS, MSI	Intestinal	NM	NM	NM
Yamaguchi *et al*[38]	Corpus, antrum	NM	NM	NM	NM	Surgery or ESD

pTNM: Pathological tumor-node-metastasis; NM: Not mentioned; ESD: Endoscopic submucosal dissection; NET: Neuroendocrine tumor; NEC: Neuroendocrine carcinoma; EBV: Epstein-Barr virus; CIN: Chromosomal instability; GS: Genomically stable; MSS: Microsatellite stable; MSI: Microsatellite instable; SRCC: Signet-ring cell cancer; GEJ: Gastroesophagus junction; HER2: Human epidermal growth factor receptor 2.

were established with a pooled success rate of 70.9% (95%CI: 49.8-88.7) (Figure 4B).

Differentiation type

Twenty-seven poorly differentiated GCOs from three studies showed a pooled successful GCO establishment rate of 64.6% (95%CI: 46.0-81.3), five moderately differentiated GCOs from two studies showed a pooled success rate of 31.0% (95%CI: 6.3-61.3), six SRCC-derived GCOs from four studies showed a pooled success rate of 32.7% (95%CI: 0.6-76.7) (Figure 4C).

pTNM cancer stage

Three pTNM I-II stage GCOs from two studies showed a pooled successful GCO establishment rate of 38.3% (95%CI: 4.1-79.1) that is lower than that for 18 pTNM stage III-IV GCOs (65.2%, 95%CI: 45.7-82.7) (Figure 4D).

Growth factors employed

All the studies included in the subgroup analysis used a range of growth factors in the culture medium, including Wnt3a, R-spondin-1, EGF, FGF10, A83-01, and Noggin. However, there were variations in the use of B27, Nutlin-3, N-acetylcysteine, gastrin, FGF-2, and Y-27632 among the retrieved studies.

A total of 103 GCOs were constructed using a culture medium containing B27, with a success rate of 68.7% (95%CI: 46.9-83.3). The success rate without using B27 was 69.5% (95%CI: 45.2-87.8), and there was no significant difference between the two groups (P = 0.94) (Figure 5A).

Table 2 The culture characteristic of included studies

Ref.	Digestion	Success/total	Morphology	Passage time	Culture medium changing time
Yan *et al*[21], 2018	EDTA, DL-dithio-threitol	34/68	NM	2 wk	Per 2-3 d
Kawasaki *et al* [22], 2020	Liberase TH, TrypLE express	3/8	NM	NM	Per 3-4 d
Nanki *et al*[23], 2018	Liberase TH, TrypLE express	44/59	Solid, glandular, mixed	NM	Every 3 or 4 d
Steele *et al*[24], 2019	Collagenase, hyalur-onidase	7/10	Spherical nest, cribriform glandular	NM	Every 2 d
Li *et al*[25], 2022	Collagenase	12/26	Glandular, solid, cystic, grape-like, or mixed	Every 2 wk	Every 3 d
Zou *et al*[26], 2022	Collagenase I, II, IV	9/10	Glandular, solid, mixed	Every 6-8 d	Every 2-3 d
Li *et al*[27], 2023	NM	12/26	Dense morphology and no lumen. Only a few showed cystic structure and epithelial thickening	Every 1-2 wk	Every 3 d
Togasaki *et al* [28], 2020	Liberase TH, TrypLE express	7	NM	NM	Every 3-4 d
Xiao *et al*[29], 2020	Collagenase	3	Cystic	Every 2-3 d	Every 4 d
Seidlitz *et al* [30], 2019	EDTA, collagenase, hyaluronidase	20	Non-coherent grape-like growth pattern, compact morphology with no lumen, a single layered epithelium and cyst-like structure	Twice a week	Twice a week
Gao *et al*[31], 2018	Collagenase II, TrypLE	15	Gastric pit cells surrounding a central lumen	Every 5-8 d	Every 2-3 d
Bartfeld *et al* [32], 2015	EDTA	10	Buddings that surrounded a central lumen	Every 14 d	Every 2-3 d
Wang *et al*[33], 2019	NM	3	NM	NM	Every 2 d
Kumar *et al*[34], 2022	Collagenase	31	NM	Every 7-10 d	NM
Harada *et al* [35], 2021	EDTA	12	Crypt-like	Twice a week	Every 3 d
Ukai *et al*[36], 2020	15 mM EDTA	10	Crypt-like	Twice a week	Every 2-3 d
Tong *et al*[37], 2023	Not accessible	56/58	NM	NM	NM
Yamaguchi *et al* [38], 2022	Collagenase type 3, DNAse I	14	Cystic	NM	NM

NM: Not mentioned.

Among the three studies that utilized N-acetylcysteine and gastrin but did not include nutlin-3, the success rate was 71.9% (95%CI: 38.2-96.2). In contrast, the other three studies that used nutlin-3 without N-acetylcysteine and gastrin had a success rate of 71.5% (95%CI: 59.3-82.3), and there was no significant difference between the two groups (P = 0.95) (Figure 5B-D).

Regarding FGF-2, three studies used it and achieved a success rate of 67.4% (95%CI: 21.6-99.2), while the other three studies that did not use FGF-2 had a success rate of 67.9% (95%CI: 49.2-84.2), with no significant difference between the groups (P = 0.98) (Figure 5E).

Two studies employed the Rho Kinase (ROCK) inhibitor Y-27632 and successfully generated 21 GCOs, resulting in a success rate of 58.2% (95%CI: 41.6-75.0). In contrast, the studies that did not use Y-27632 generated 137 GCOs with a success rate of 70.0% (95%CI: 39.8-93.3). However, there was no statistically significant difference between the two groups (P = 0.505) (Figure 5F).

Digestive enzymes used

Two studies used Liberase TH and TrypLE for digestion, resulting in a pooled success rate of 71% (95%CI: 59.3-82.3). Three studies used Collagenase for digestion, yielding a pooled success rate of 72.1% (95%CI: 44.4-93.6, P = 0.04). The use

Table 3 The growth factor applied in the gastric cancer organoids culture

Ref.	Wnt	R-spondin-1	Noggin	B27	EGF	FGF-10	N-acetylcystenine	Gastrin	A83-01	FGF-2	IGF-1	Nicotinamide	Noggin	Nutlin-3	Y-27632	HGF	Other
Tong *et al*[37]	+	+	+	+	+	+	+	+	+	+			+				
Steele *et al*[24]	+	+	+		+	+	+	+	+				+		+	+	SB202190, p38, MAPK inhibitor, prostaglandin E2
Nanki *et al*[23]	+	+	+		+	+			+				+	+			
Yan *et al*[21]	+	+	+	+	+	+	+	+	+	+			+				
Li *et al*[25]	+	+	+	+	+	+	+	+	+			+	+		+		
Kawasaki *et al*[22]	+	+	+		+	+			+	+	+		+	+		+	GDNF, DAPTγ-secretase inhibitor and Palbociclib CDK4/CDK6 selective inhibitor
Togasaki *et al*[28]	+	+	+		+	+			+								
Seidlitz *et al*[30]	+	+	+	+	+	+	+	+	+			+					
Gao *et al*[31]	+	+	+		+	+		+	+			+					Hamburg, TGF, rho-associated
Bartfeld *et al*[32]	+	+	+		+	+		+	+			+					Hamburg, TGF, rho-associated
Wang *et al*[33]	+	+	+		+	+			+			+	+		+	+	Prostaglandin E2, SB202190
Kumar *et al*[34]	+	+	+		+	+		+	+			+	+		+	+	Prostaglandin E2, SB202190
Harada *et al*[35]	+	+	+	+	+	+	+		+			+	+		+		SB203580
Ukai *et al*[36]	+	+	+	+	+	+	+		+			+	+		+		SB203580
Yamaguchi *et al*[38]	+	+		+	+	+	+	+				+			+		TGF-βi

EGF: Epidermal growth factor; FGF: Fibroblast growth factor; IGF-1: Insulin-like growth factors-1; HGF: Hepatocyte growth factor; GDNF: Glialcellline-derived neurotrophic factor; TGF: Transforming growth factor.

of EDTA for digestion showed a success rate of 50.0% (95%CI: 37.6-62.4) that was statistically significant (P = 0.04) (Figure 6).

DISCUSSION

GCOs are capable of simulating a range of *in vivo* tumor biological behaviors within *in vitro* research models, such as tumorigenesis, molecular signaling pathway transduction, antitumor drug screening, and targeted therapy for patients with tumors. Seidlitz *et al*[30] and Steele *et al*[24] found that the morphological characteristics and gene expression within

Table 4 The quality assessment according to our adjusted Methodological Index for Non-Randomized Studies tool

Criteria	Tong *et al* [37], 2023	Steele *et al* [24], 2019	Nanki *et al* [23], 2018	Yan *et al* [21], 2018	Li *et al*[25], 2022	Kawasaki *et al* [22], 2020	Li *et al*[27], 2023	Zou *et al* [26], 2022
A clearly stated aim	2	2	2	2	2	2	2	2
Inclusion of consecutive patients	2	2	2	2	2	2	2	2
Prospective collection of data	2	2	2	2	2	2	2	2
Endpoints appropriate to the aim of the study	2	2	2	2	2	2	2	2
Follow-up period appropriate to the aim of the study	2	1	2	2	1	2	2	1
Loss to follow up less than 5%	2	1	2	2	2	2	2	1
Baseline equivalence of groups	2	2	2	2	1	1	1	2
Adequate statistical analyses	2	2	2	2	2	2	2	2
Total	16	15	16	16	14	15	15	14

The items are scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate). Criteria: (1) A clearly stated aim: The question addressed should be precise and relevant in the light of available literature; (2) Inclusion of consecutive patients: All patients potentially fit for diagnostic criteria of gastric cancer have been included in the study during the study period; (3) Prospective collection of data: Data were collected according to a protocol established before the beginning of the study; (4) Endpoints appropriate to the aim of the study: Unambiguous explanation of the criteria of successfully culture; (5) Follow-up period appropriate to the aim of the study: The follow-up should be sufficiently long at least for 30 d to allow the assessment of the stable construction; (6) Loss to follow up less than 5%: All gastric cancer organoids should be included in the follow up. Otherwise, the proportion fail to follow up should not exceed the proportion experiencing the major endpoint; (7) Baseline equivalence of groups: The groups should be similar regarding the criteria other than the studied endpoints. Absence of confounding factors that could bias the interpretation of the results; and (8) Adequate statistical analyses: The statistics were in accordance with the type of study.

GCOs were similar to those of the primary tissue. GCOs can mimic typical human GC characteristics and altered signaling pathways, demonstrating their role as sentinels of the response to cancer treatments. The highly altered genetic background of individual patients with cancer often hinders an accurate prognosis because differences in the status of various signaling pathways can interfere with each other. Yan *et al*[21] discovered differentially expressed genes between tumor organoids and paired tumor tissues, and most of those were highly expressed in cancer tissues. After two rounds of ComBat batch deletion, the cultured organoids retained the gene sequences of the cancer cells *in vivo*. This organoid biobank covers nearly all the known molecular subtypes and subtype-specific mutational profiles. A mixture of GC and normal tissue was found in primary GCOs in the study by Nanki *et al*[23]. Whole-exome sequencing, copy number analysis, and MSI analyses were performed to determine gene expression. Methylation microarray analysis revealed that the gene expression and DNA methylation patterns of GCs could be accurately determined regardless of tumor purity in the original specimen. Engineered organoids have also been used to explore the CDH1/TP53 loss-mediated Ri phenotype. Kumar *et al*[34] compared the single-cell profiles of patient-derived organoids (PDOs) and primary tumors using single-cell sequencing. GCOs exhibited an upregulation of cancer-related modular genes compared to normal PDO epithelial cells. Primary tissues and PDOs also differed in cell clusters, with enriched epithelial and stromal clusters and depleted lymphoid and plasma clusters. Gene expression comparisons between PDOs and primary tissues showed that plasma cells showed the largest differences in gene expression profiles, whereas epithelial cells were relatively more conserved in their characteristics. The largest tumor-associated gene expression differences in tumor epithelial cell components may come from autologous cells and enterocytes.

To our knowledge, this is the first systematic analysis and meta-analysis of the establishment rates of GCOs. The culture method for GCOs was similar to that used for ORISC-derived organoids[39]. Briefly, the necrotic components of the tumor and normal tissues were removed, digested into single cells and cultured in Matrigel to form a 3D structure. The necessary growth factors and nutritional support are then supplied to eventually form organoids[39,40]. However, the current success rate of GCO culture remains low. Our results showed that the pooled success rate of the eight studies was 66.6% (95%CI: 0.468-0.840, I^2 = 88.77%). A possible source of heterogeneity may be the small sample size. These stable and elevated success rates are a fundamental requirement for genetic studies, biomarker identification, drug screening, and preclinical evaluation of personalized medical regimens[41]. Tumor cells cultured *in vitro* grow and form organoids through cell division and proliferation; however, proliferating yet non-tumor cells can also form organoids and tend to overgrow by applying a growth advantage that has a greater impact on the growth of tumor organoids. Nevertheless, the reason for the growth advantage of non-tumor cells is unclear, and the prevailing speculation is that tumor cells have a

Table 5 The gastric cancer organoids construction rate from different subgroups

Subgroup	Successful cases	Number of total	Number of study	Success rate (%)	95%CI	*P* value	I^2
Gender							
Female	14	23	4	67.0	31.1-95.7	0.31	36.07
Male	22	47	4	46.7	31.5-62.2		
Tissue source							
Endoscopic biopsy	16	32	3	53.7	27.1-79.5	0.28	37.35
Surgery and ESD	28	40	4	70.9	49.8-88.7		
Differentiate type							
Poor	27	43	3	64.6	46.0-81.3	0.106	42.54
Moderate	5	15	2	31.0	6.3-61.3		
SRCC	6	16	4	32.7	0.6-76.7		
pTNM							
I-II	3	8	2	38.3	4.1-79.1	0.108	42.54
III-IV	18	28		65.2	45.7-82.7		
Enzyme							
Liberase TH, TrypLE	47	67	2	71.5	59.3-82.3	0.04	69.27
EDTA, DL-dithiothreitol	34	68	1	50.0	37.6-62.4		
Collagenase	30	46	3	72.1	44.4-93.6		
Growth factor							
B27	103	152	3	68.7	46.9-83.3	0.94	90.53
Non-B27	55	77	3	69.5	45.2-87.8		
N-acetylcysteine	111	162	4	71.9	38.2-96.2	0.95	90.52
Non-N-acetylcysteine	47	67	2	71.5	59.3-82.3		
Gastrin	111	162	4	71.9	38.2-96.2	0.95	90.53
Non-gastrin	47	67	2	71.5	59.3-82.3		
FGF-2	93	134	3	67.4	21.6-99.2	0.98	90.53
Non-FGF-2	65	95	3	67.9	49.2-84.2		
Nutlin-3	47	67	2	71.5	59.3-82.3	0.95	90.53
Non-nutlin-3	111	162	4	71.9	38.2-96.2		
Y-27632	21	36	2	58.2	41.6-75.0	0.505	90.53
Non-Y-27632	137	193	4	70.0	39.8-93.3		

pTNM: Pathological tumor-node-metastasis; CI: Confidence interval; ESD: Endoscopic submucosal dissection; SRCC: Signet-ring cell cancer; FGF-2: Fibroblast growth factor-2.

higher mitotic failure rate than normal cells, resulting in increased tumor cell death[42]. Another speculation is that there may be many recessive mutations in seemingly normal tissues at the edges of cancer tissue, and these recessive mutations give seemingly normal cells a growth advantage over tumor cells[43,44]. To reduce these effects, researchers have proposed several solutions for removal of the contaminating normal cells. First, certain cytokines or small molecule inhibitors such as A83-01 (transforming growth factor-β inhibitor) were added or reduced during organoid culture to screen for non-tumor organoids carrying targeting-dependent mutations[42]. Studies have shown that malignant lesions caused by mutations in the p53 pathway are more prevalent in GC[45]; thus, studies have used nutlin-3 to create pure tumor organoids. Nutlin-3 is a small molecule inhibitor of the E3 ubiquitin ligase MDM2, that stabilizes TP53 expression by disrupting the binding of TP53 to its negative regulator MDM2[42]. Notably, the ROCK inhibitor, Y-27632, plays an important role in non-tumor cells by reducing apoptosis and promoting proliferation. Therefore, the proportion of GCOs with dysregulated RHO proteins can also be increased by using ROCK inhibitor-free medium to exclude non-tumor carcinoids[46]. However, these approaches have some limitations, because not all GCs develop through a specific

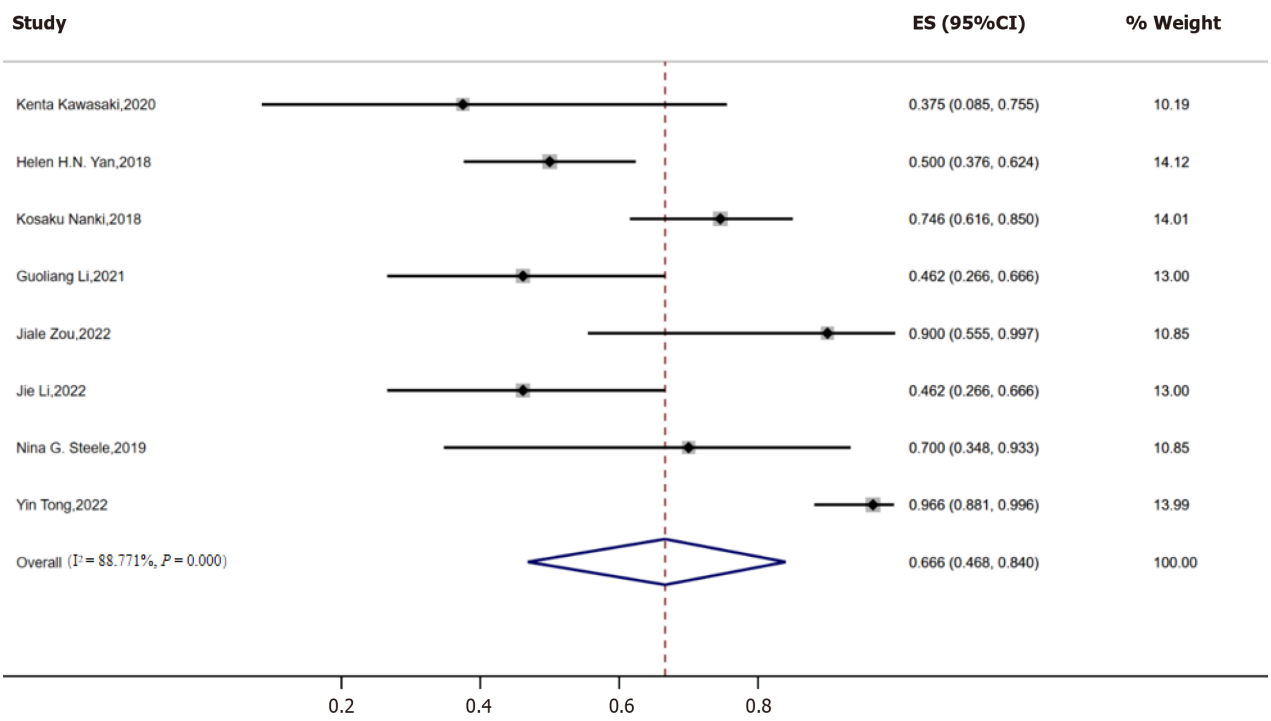


Figure 2 A forest plot showing the pooled successful gastric cancer organoid culture rate. The plot indicates a pooled success rate of 66.6% with a 95% confidence interval: 0.468-0.840, *I2* = 88.77%. ES: Effect size; CI: Confidence interval.

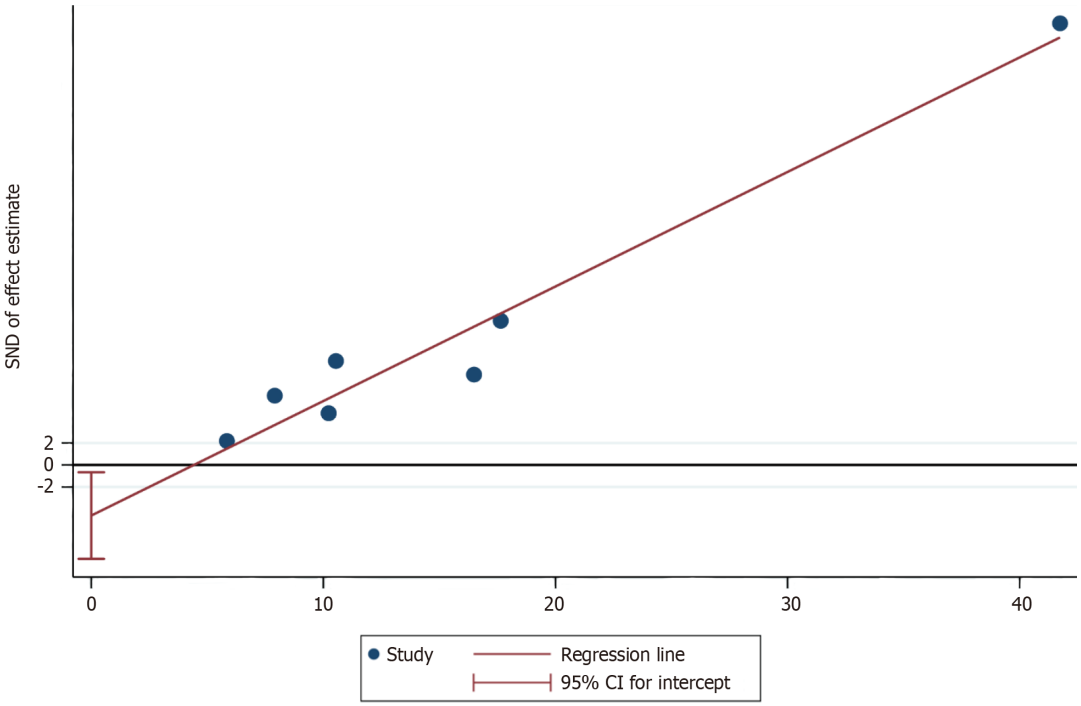
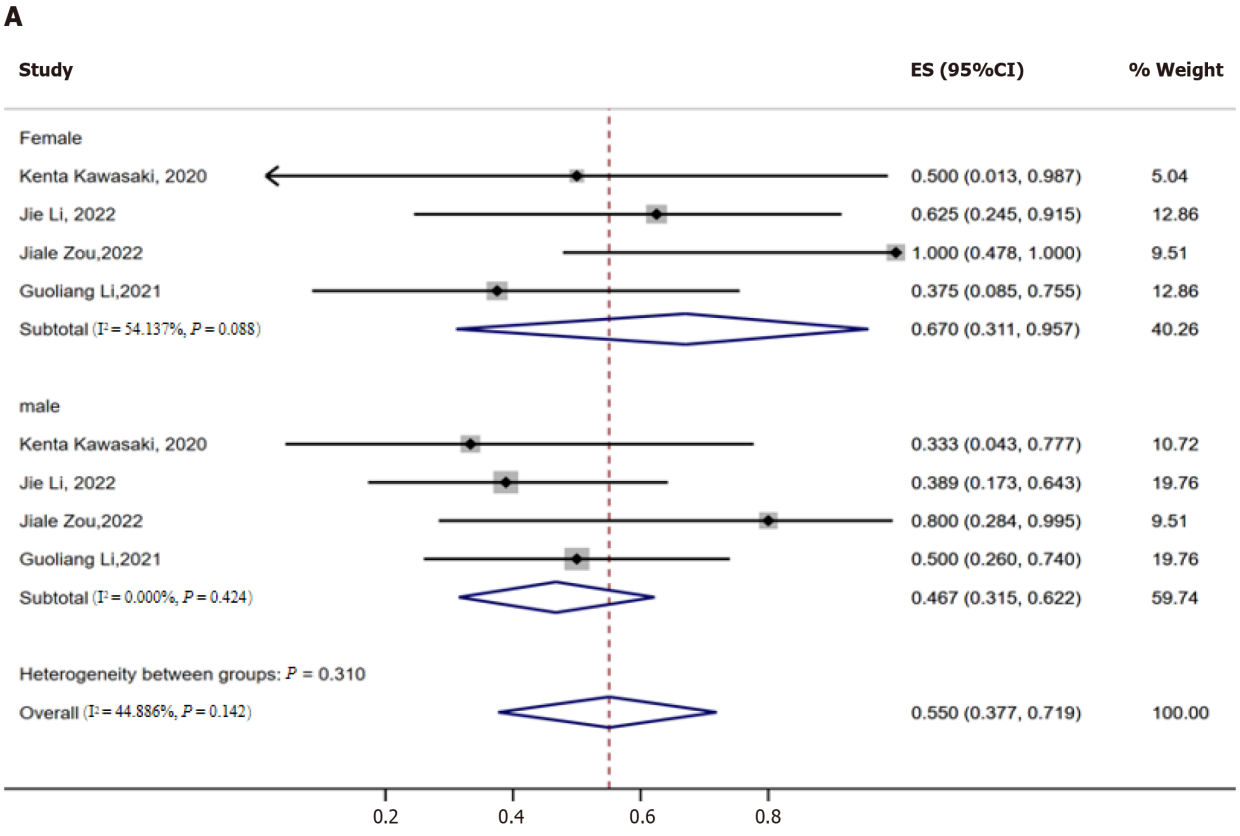
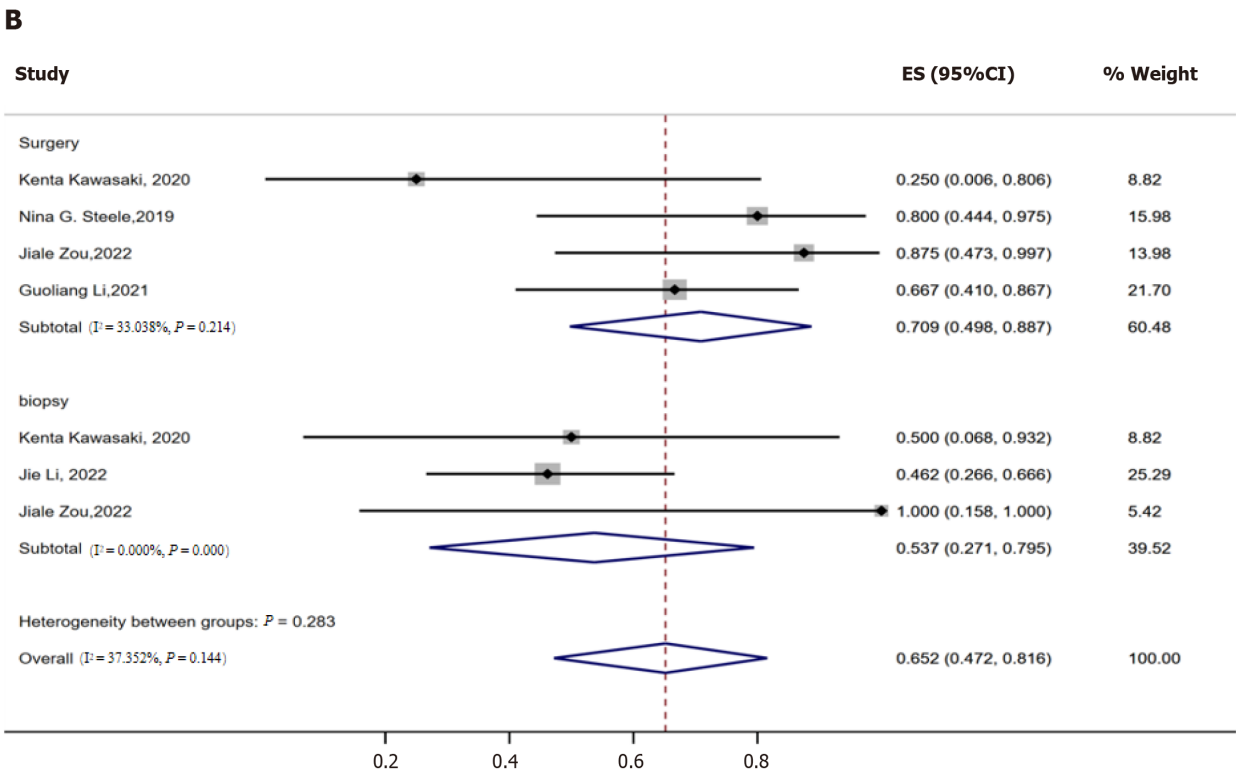


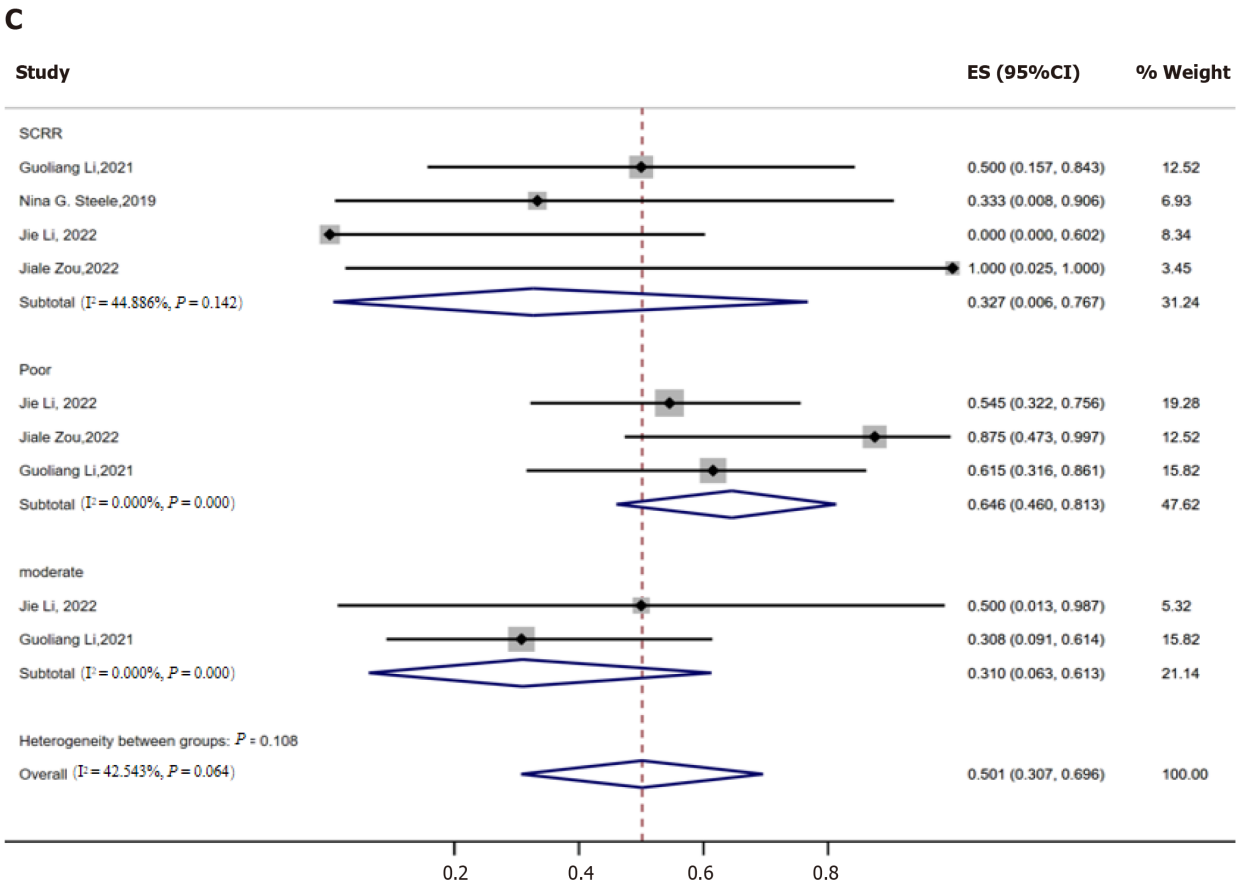
Figure 3 The funnel plot showed the presence publication bias. The funnel plot showed the existence of publication bias. CI: Confidence interval; SND: Standardized normal deviate.

pathway, and the withdrawal of a factor alone may lead to the death of some GCOs while removing normal organoids. In addition, our subgroup analysis revealed that the construct success rate of GCOs was also influenced by other factors such as tissue source, pathological histology, sex, and pTNM cancer stage.

Our findings showed a lower success rate for tissues from endoscopic biopsy than for those from ESD or surgical specimens. The opening size of the biopsy forceps is approximately 6.8-8 mm, and this yields much smaller tissue than ESD or surgery[47]. The size of the gastroscopic sample is closely related to the depth, and if the specimen is superficial and small, an endoscopic biopsy may result in pathological findings that are inconsistent with the actual situation[48].


A
Study
ES (95%CI)
% Weight
Female
Kenta Kawasaki, 2020
0.500 (0.013, 0.987)
5.04
Jie Li, 2022
0.625 (0.245, 0.915)
12.86
Jiale Zou,2022
1.000 (0.478, 1.000)
9.51
Guoliang Li,2021
0.375 (0.085, 0.755)
12.86
Subtotal (I² = 54.137%, P = 0.088)
0.670 (0.311, 0.957)
40.26
male
Kenta Kawasaki, 2020
0.333 (0.043, 0.777)
10.72
Jie Li, 2022
0.389 (0.173, 0.643)
19.76
Jiale Zou,2022
0.800 (0.284, 0.995)
9.51
Guoliang Li,2021
0.500 (0.260, 0.740)
19.76
Subtotal (I² = 0.000%, P = 0.424)
0.467 (0.315, 0.622)
59.74
Heterogeneity between groups: P = 0.310
Overall (I² = 44.886%, P = 0.142)
0.550 (0.377, 0.719)
100.00
0.2
0.4
0.6
0.8


B
Study
ES (95%CI)
% Weight
Surgery
Kenta Kawasaki, 2020
0.250 (0.006, 0.806)
8.82
Nina G. Steele,2019
0.800 (0.444, 0.975)
15.98
Jiale Zou,2022
0.875 (0.473, 0.997)
13.98
Guoliang Li,2021
0.667 (0.410, 0.867)
21.70
Subtotal (I² = 33.038%, P = 0.214)
0.709 (0.498, 0.887)
60.48
biopsy
Kenta Kawasaki, 2020
0.500 (0.068, 0.932)
8.82
Jie Li, 2022
0.462 (0.266, 0.666)
25.29
Jiale Zou,2022
1.000 (0.158, 1.000)
5.42
Subtotal (I² = 0.000%, P = 0.000)
0.537 (0.271, 0.795)
39.52
Heterogeneity between groups: P = 0.283
Overall (I² = 37.352%, P = 0.144)
0.652 (0.472, 0.816)
100.00
0.2
0.4
0.6
0.8



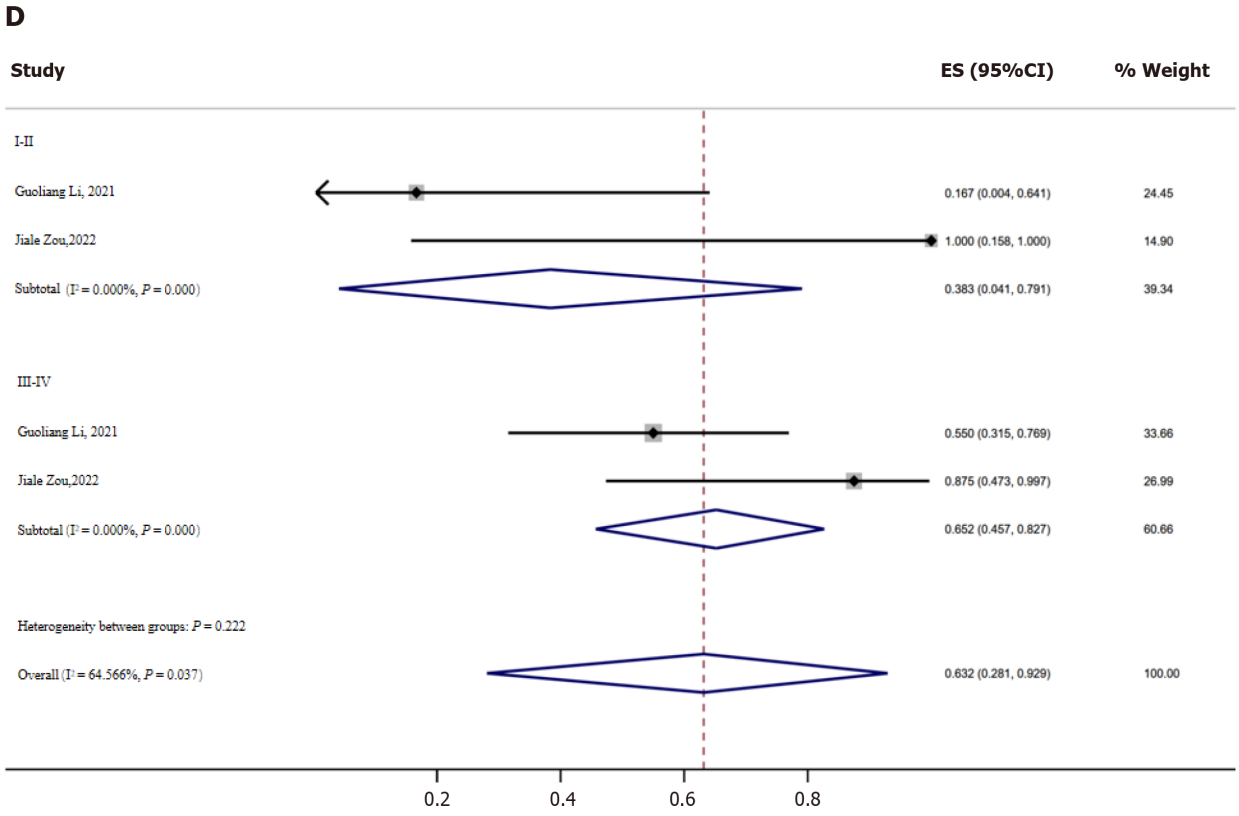


Figure 4 Forest plot of the pooled successful gastric cancer organoids culture rate according to different subgroups. A: Gastric cancer organoids (GCOs) from female and male showed the success rate of 67.0, 95% confidence interval (CI): 31.1-95.7, and 46.7%, 95%CI: 31.5-62.2; B: GCOs from surgery or endoscopic submucosal dissection and biopsy showed a pooled success rate of 70.9%, 95%CI: 49.8-88.7 and 53.7%, 95%CI: 27.1-79.5; C: GCOs of poor-differentiated, moderate-differentiated and signet-ring cell cancer showed a pooled success establishment rate of 64.6, 95%CI: 46.0-81.3, 31.0, 95%CI: 6.3-61.3 and 32.7, 95%CI: 0.6-76.7; D: GCOs with pathological tumor-node-metastasis (pTNM) I-II and pTNM stage III-IV stage showed a pooled success establishment rate of

38.3, 95%CI: 4.1-79.1 and 65.2, 95%CI: 45.7-82.7. ES: Effect size; CI: Confidence interval.

The pooled successful construct rate of GCOs obtained from poorly differentiated cancers was higher than that obtained from moderately differentiated cancers and SRCC. The Japanese Classification of Gastric Carcinoma classifies GC into differentiated and undifferentiated types, based on the World Health Organization classification[49]. Among the undifferentiated types, there is a group of adenocarcinomas with few glandular structures that are histologically diagnosed as poorly differentiated adenocarcinomas[50]. Poorly differentiated cancer has been suggested to be a relevant prognostic factor associated with perineural invasion, lymph node metastasis, and poor prognosis[51]. According to the Lauren staging system, GC can be divided into intestinal, diffuse, and mixed type[52,53]. The characteristics of intestinal-type GC are a better differentiated morphology, often forming glandular ducts, larger mucous vacuoles at the top of cancer cells, sometimes forming cup-shaped cells, microvilli on the surface, and more mucus in the glandular lumen. The cytoplasm may contain a highly active aminopeptidase unique to intestinal epithelial cells, and this type of GC is often accompanied by intestinal epithelial hyperplasia. Diffuse cancer cells are poorly differentiated gastric mucosal cells. Cancer cells are often scattered or grow in small clusters to infiltrate the surrounding tissues and rarely form glandular cavities. Compared to intestinal cancers, diffuse gastric carcinomas have a different type of fibrosis that reduces elasticity and compliance. It is speculated that cancer cells release a factor that stimulates this process, and this indicates that the two kinds of GC have different origins[54-56]. Contamination with epithelial cells and scarcity of cancer cells are the main challenges in GCOs culture[21,57]. The construction rate difference between poorly differentiated and moderately differentiated types of GCOs may be due to the number of cancer cells since poorly differentiated GC is more malignant. Previous studies showed that patients with intestinal-type GC were older and there were more men than women. By comparison, patients with diffuse GC are younger and mostly women[58]. This characteristic may explain the reason for the higher construction rate of the GCOs derived from women participants.

When mucinous adhesion proteins fill over 50% of the entire cell and push the nucleus to one side, resembling a ring, it is called a SRCC[59]. The presence of these adhesion proteins may make SRCCs unique in terms of tumorigenesis, development and treatment[60,61]. SRCC organoids are difficult to establish because signet ring cells are closely associated with stromal cells in the tumor, and it is extremely difficult to isolate and enrich them from a large number of tumor-associated fibroblasts. In addition, there is a period of stagnation before the rapid growth and proliferation of SRCC that can lead to cell death in improper culture[62].

Gastroenteropancreatic neuroendocrine neoplasms are rare, heterogeneous tumors comprising well-differentiated neuroendocrine tumors and poorly differentiated NEC. Kawasaki *et al*[22] established three NEC GCOs with a success rate of 37.8%. Most gastroenteropancreatic neuroendocrine neoplasm organoids grow independently of Wnt/R-spondin and EGF, regardless of the lack of associated driver mutations.

GCOs at pTNM stages III-IV showed higher culture success rates than those at stages I-II. T-stage refers to tumor infiltration. A higher T-grade indicates deeper tumor infiltration and a higher TNM cancer stage. However, a correlation between the culture rate of GCOs and TNM cancer stage had not been studied. By combining the abovementioned findings with the results of our subgroup analysis of the pathological type, we speculate that the more advanced the cancer cell stage is, the better the cell quality and the higher the GCOs culture success rate will be.

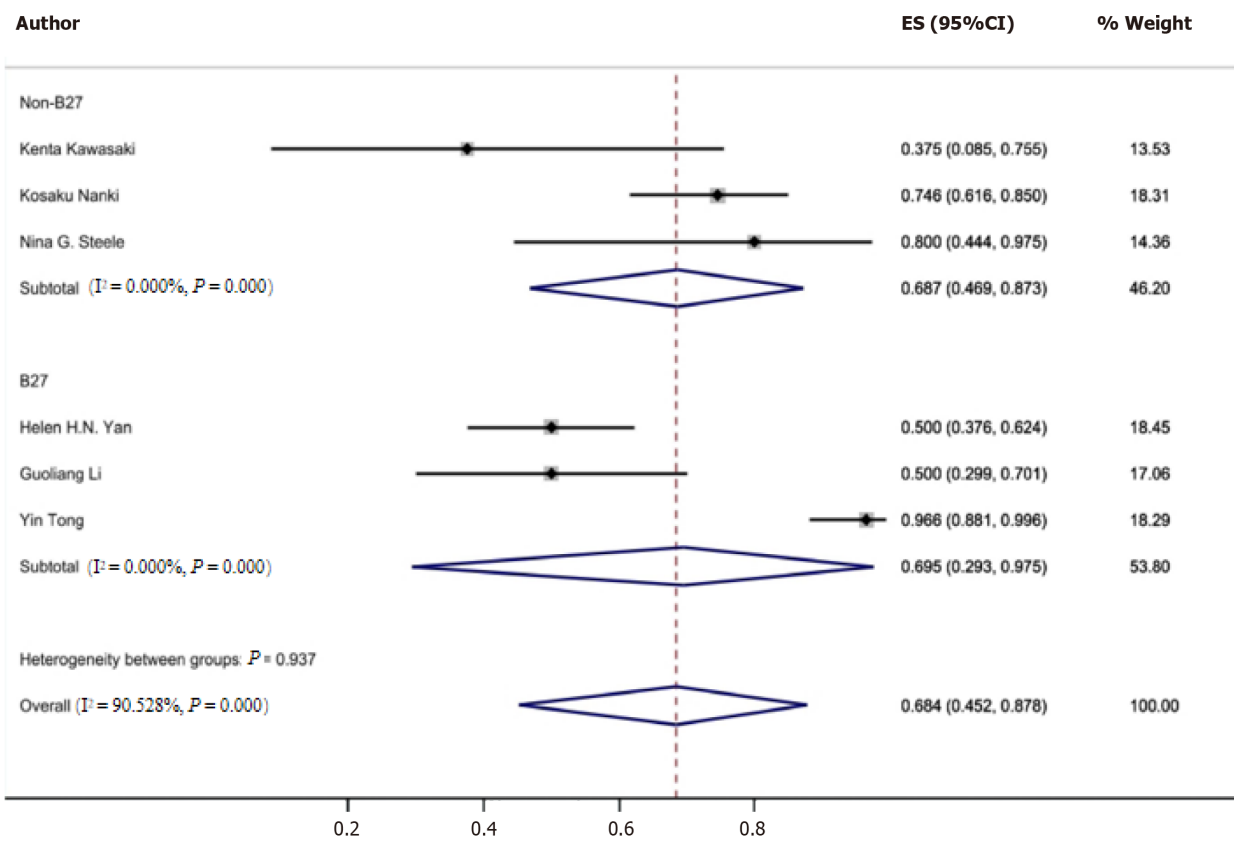
However, the different tumor locations of GCOs seem to lead to a separate construction rate, although there is not enough research data to pool the results. Li *et al*[27] reported successful GCO culture rates of 33% and 60% in the antrum and corpus, respectively. Previous Studies have found that most patients with antral gastritis show mucosal erythema, erosion, or ulcers. Histology also suggests chronic inflammation even in cases of normal mucosa retrieved *via* endoscopy [63]. The low culture rate of antral GCOs may be related to antral inflammation and edema.

Nonetheless, our study has several limitations. First, the sample size of every included study was generally small, varying from 8 to 68, and this may have led to publication bias. It is difficult to achieve a reliable result from a meta-analysis with such a small sample size because GCOs are still difficult to establish stably, and several studies have not reported detailed GCOs construction information. However, our initial results are meaningful in stating the currently reported culture rates and possible factors for GCOs establishment. Second, basic GC biology and heterogeneity have been better understood through the rapid development of sequencing technology. The Cancer Genome Atlas group[64] and the Asian Cancer Research Group[65] provided the basis for the molecular classification of GC, such as MSI, microsatellite stable (MSS)/EMT, MSS/TP53+), or MSS/TP53-, according to the genomic mutation. This classification not only reflects the mechanism of GC development but also serves as an effective tool for targeted therapy. This opens new perspectives for the treatment of GC, such as the combination of emerging immunotherapies with molecularly targeted drugs, to select the most appropriate and precise therapies for patients with advanced GC. Construction of GCOs based on a molecular classification is important for drug screening and mechanistic research. Only six studies recorded the molecular characteristics of GCOs; however, they did not provide all the culture sample information, regardless of whether it was constructed successfully or not.

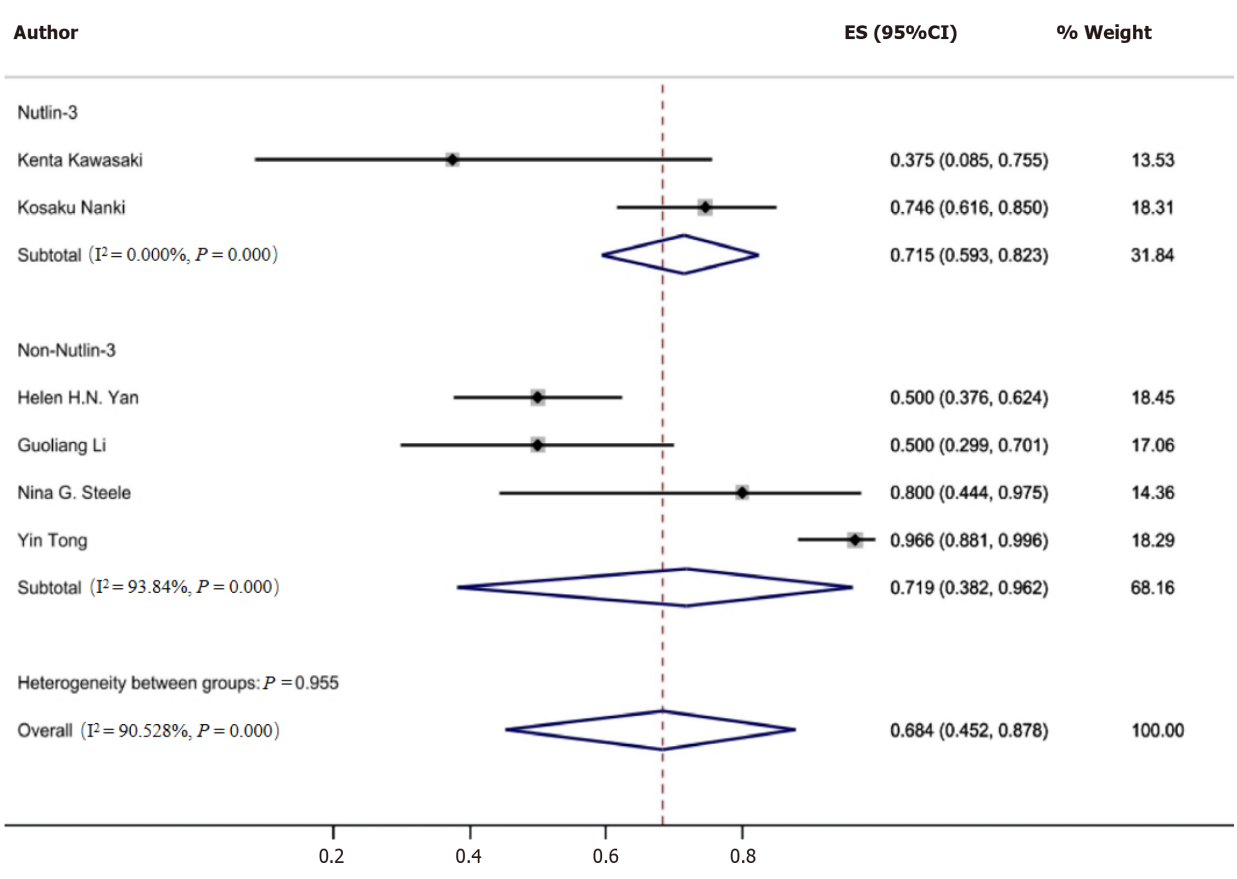
CONCLUSION

Currently, the success rate of GCO culture requires improvement. The construction success rate of the GCOs derived from women was higher than that of GCOs derived from men. GCOs obtained through surgery and ESD have a higher success rate. The GCOs with a lower degree of differentiation had a relatively higher success rate. GCOs with pTNM

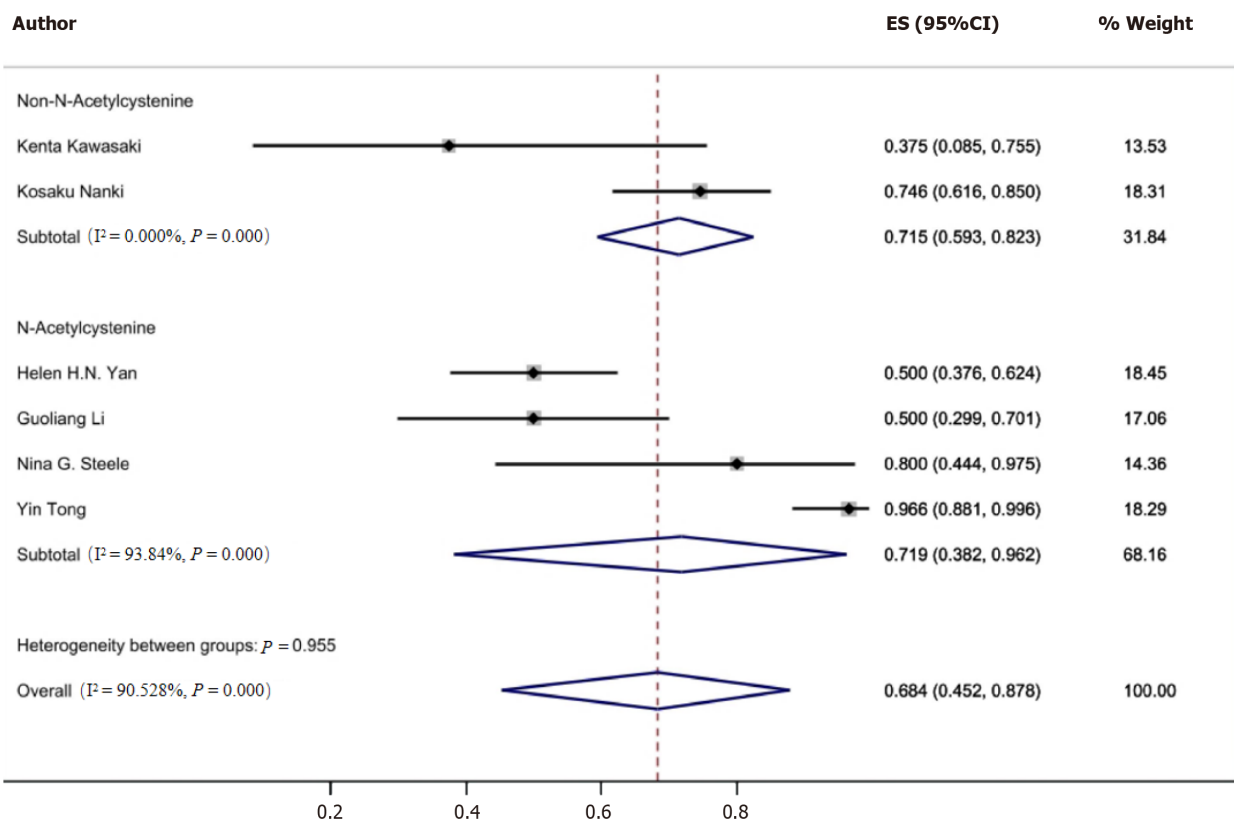
A


Author
ES (95%CI)
% Weight
Non-B27
Kenta Kawasaki
0.375 (0.085, 0.755)
13.53
Kosaku Nanki
0.746 (0.616, 0.850)
18.31
Nina G. Steele
0.800 (0.444, 0.975)
14.36
Subtotal (I² = 0.000%, P = 0.000)
0.687 (0.469, 0.873)
46.20
B27
Helen H.N. Yan
0.500 (0.376, 0.624)
18.45
Guoliang Li
0.500 (0.299, 0.701)
17.06
Yin Tong
0.966 (0.881, 0.996)
18.29
Subtotal (I² = 0.000%, P = 0.000)
0.695 (0.293, 0.975)
53.80
Heterogeneity between groups: P = 0.937
Overall (I² = 90.528%, P = 0.000)
0.684 (0.452, 0.878)
100.00
0.2
0.4
0.6
0.8

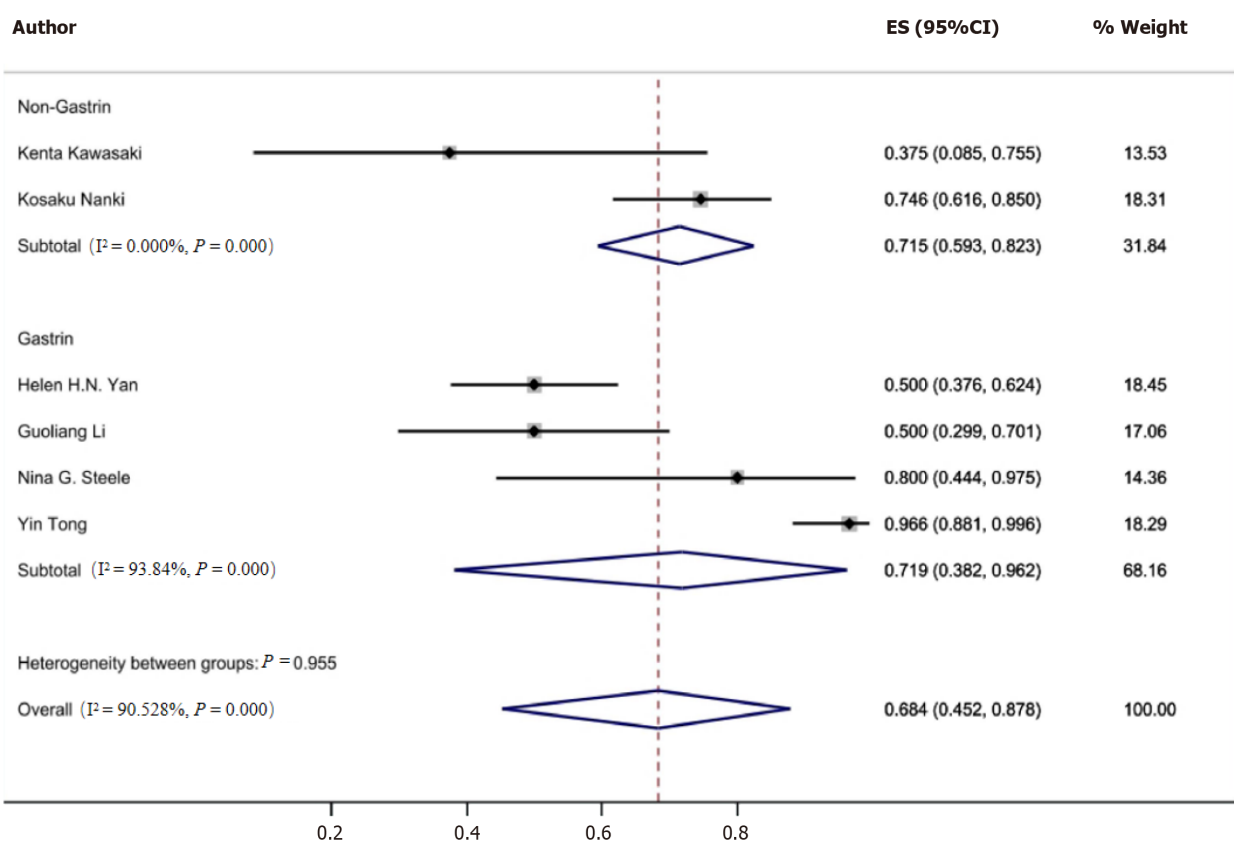
B


Author
ES (95%CI)
% Weight
Nutlin-3
Kenta Kawasaki
0.375 (0.085, 0.755)
13.53
Kosaku Nanki
0.746 (0.616, 0.850)
18.31
Subtotal (I² = 0.000%, P = 0.000)
0.715 (0.593, 0.823)
31.84
Non-Nutlin-3
Helen H.N. Yan
0.500 (0.376, 0.624)
18.45
Guoliang Li
0.500 (0.299, 0.701)
17.06
Nina G. Steele
0.800 (0.444, 0.975)
14.36
Yin Tong
0.966 (0.881, 0.996)
18.29
Subtotal (I² = 93.84%, P = 0.000)
0.719 (0.382, 0.962)
68.16
Heterogeneity between groups: P = 0.955
Overall (I² = 90.528%, P = 0.000)
0.684 (0.452, 0.878)
100.00
0.2
0.4
0.6
0.8

C


Author
ES (95%CI)
% Weight
Non-N-Acetylcystenine
Kenta Kawasaki
0.375 (0.085, 0.755)
13.53
Kosaku Nanki
0.746 (0.616, 0.850)
18.31
Subtotal (I² = 0.000%, P = 0.000)
0.715 (0.593, 0.823)
31.84
N-Acetylcystenine
Helen H.N. Yan
0.500 (0.376, 0.624)
18.45
Guoliang Li
0.500 (0.299, 0.701)
17.06
Nina G. Steele
0.800 (0.444, 0.975)
14.36
Yin Tong
0.966 (0.881, 0.996)
18.29
Subtotal (I² = 93.84%, P = 0.000)
0.719 (0.382, 0.962)
68.16
Heterogeneity between groups: P = 0.955
Overall (I² = 90.528%, P = 0.000)
0.684 (0.452, 0.878)
100.00
0.2
0.4
0.6
0.8

D


Author
ES (95%CI)
% Weight
Non-Gastrin
Kenta Kawasaki
0.375 (0.085, 0.755)
13.53
Kosaku Nanki
0.746 (0.616, 0.850)
18.31
Subtotal (I² = 0.000%, P = 0.000)
0.715 (0.593, 0.823)
31.84
Gastrin
Helen H.N. Yan
0.500 (0.376, 0.624)
18.45
Guoliang Li
0.500 (0.299, 0.701)
17.06
Nina G. Steele
0.800 (0.444, 0.975)
14.36
Yin Tong
0.966 (0.881, 0.996)
18.29
Subtotal (I² = 93.84%, P = 0.000)
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68.16
Heterogeneity between groups: P = 0.955
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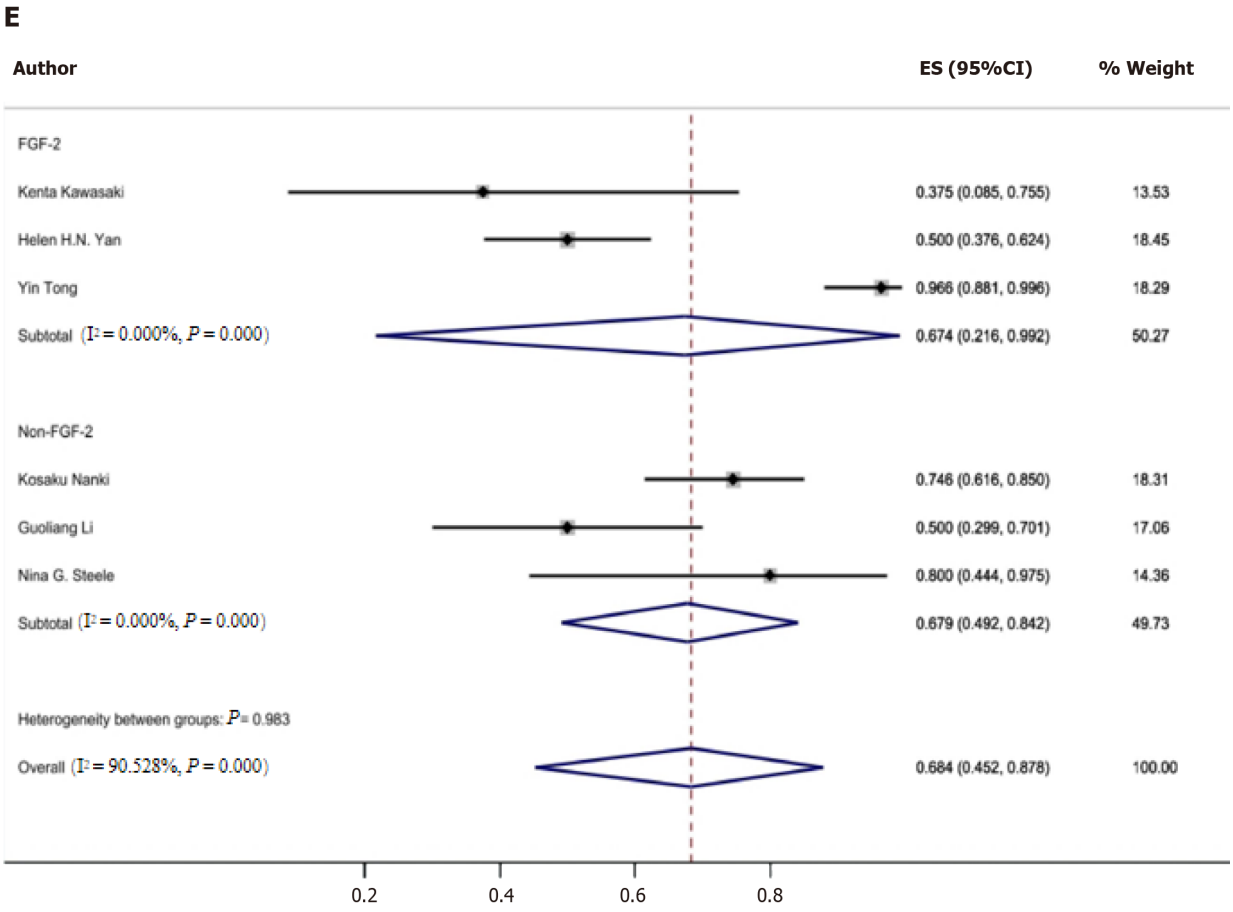
0.684 (0.452, 0.878)
100.00
0.2
0.4
0.6
0.8



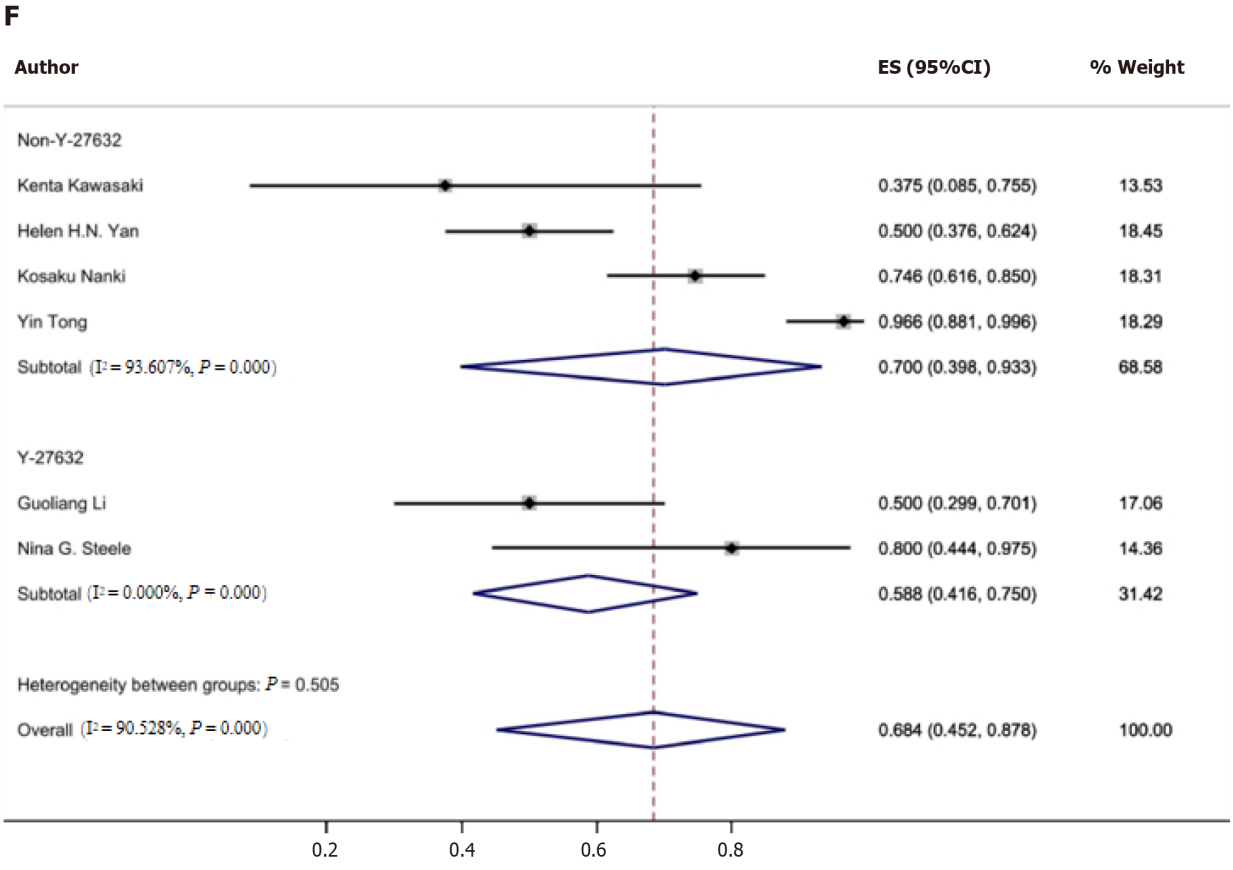


Figure 5 Forest plot of the pooled successful gastric cancer organoids culture rate according to different growth factor. A: Success rates with and without B27 supplementation: B27 group [68.7%, 95% confidence interval (CI): 46.9-83.3] *vs* non-B27 group (69.5%, 95%CI: 45.2-87.8) (*P* = 0.94); B-D:

Success rates of gastric cancer organoids (GCOs) with N-acetylcystenine and gastrin but without nutlin-3 (71.9%, 95%CI: 38.2-96.2) compared to GCOs with nutlin-3 but without n-acetylcystenine and gastrin (71.5%, 95%CI: 59.3-82.3) (P = 0.95); E: Success rates of GCOs with fibroblast growth factor-2 (FGF-2) (67.4%, 95%CI: 21.6-99.2) compared to GCOs without FGF-2 (67.9%, 95%CI: 49.2-84.2) (P = 0.98); F: Success rates of GCOs with Y-27632 (58.2%, 95%CI: 41.6-75.0) compared to GCOs without Y-27632 (70.0%, 95%CI: 39.8-93.3) (P = 0.505). ES: Effect size; CI: Confidence interval; FGF-2: Fibroblast growth factor-2.

stage III-IV had higher success rates than those with stages I-II. The use of B27, N-acetylcysteine, gastrin, nutlin-3, and FGF-2 did not significantly affect the success rate. The omission of Y-27632 enhanced the success rate. The use of Liberase TH and TrypLE or collagenase for digestion showed a higher success rate, whereas the use of EDTA for digestion showed a lower success rate, and this difference was statistically significant. More advanced culture methods and studies are required to improve the establishment rates of GCOs.

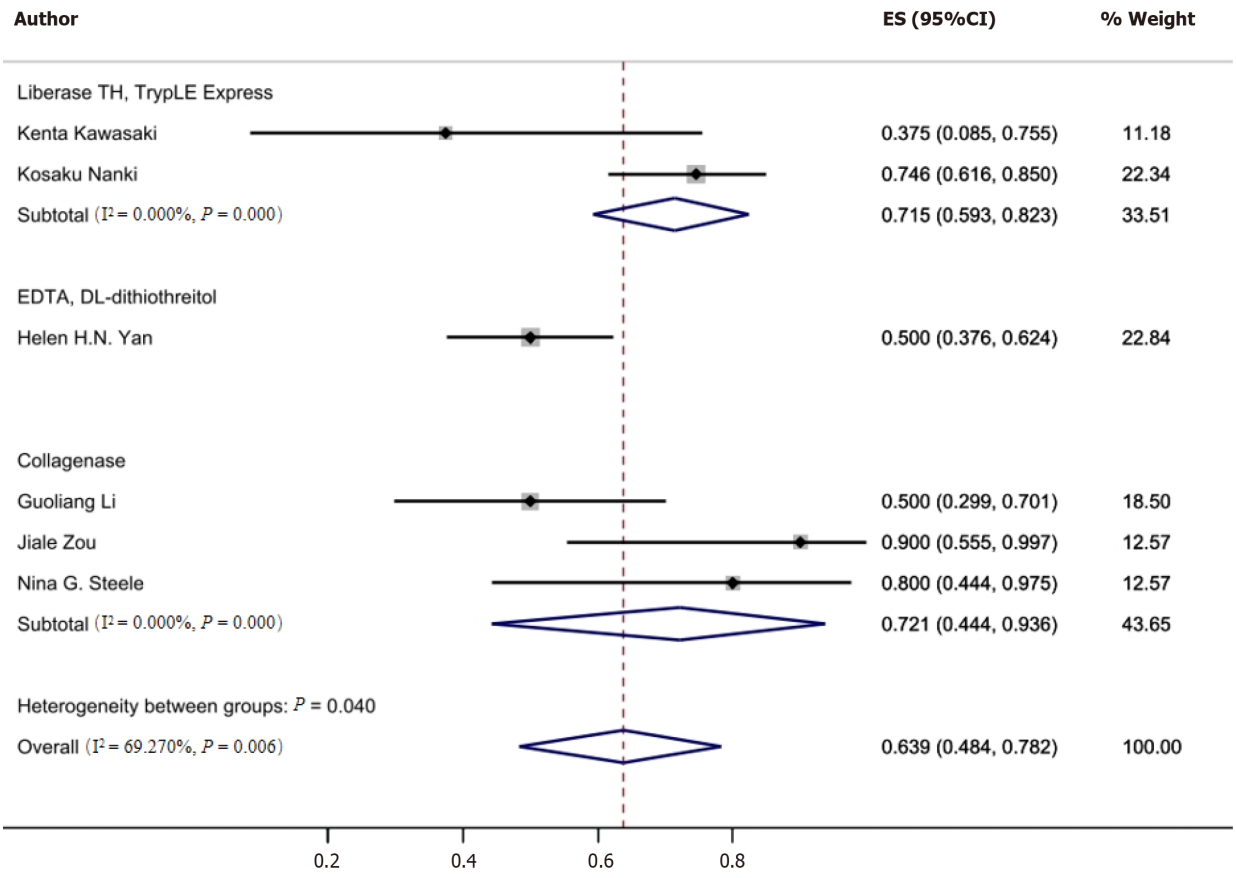


Figure 6 Forest plot of the pooled successful gastric cancer organoids culture rate according to different digestive enzyme. Liberase TH and TrypLE digestion: Success rate 71% [95% confidence interval (CI): 59.3-82.3]. Collagenase digestion: Success rate 72.1% (95%CI: 44.4-93.6, P = 0.04). EDTA digestion: Success rate 50.0% (95%CI: 37.6-62.4, P = 0.04). ES: Effect size; CI: Confidence interval.

ARTICLE HIGHLIGHTS

Research background

The study explores success rate of human-derived gastric cancer organoids (GCOs) culture, highlighting their widespread use in research and factors that influence culture success rate.

Research motivation

The study aims to review the success rates of GCO culture through a meta-analysis and explore the factors affecting these rates, addressing a significant gap in gastric cancer (GC) research.

Research objectives

The primary objective is to systematically review and meta-analyze the success rates of GCOs, identifying influencing factors that can guide future research in this area.

Research methods
The study employed a systematic review and meta-analysis, utilizing databases like PubMed, Web of Science, and EMBASE for data collection, and STATA 17.0 for meta-analysis.

Research results
The research revealed a pooled success rate of 66.6% for GCO culture, influenced by factors like sex, tissue source, and cancer stage. The study also highlighted the variation in success rates based on different methodological approaches.

Research conclusions
The study proposes new insights into the factors influencing GCO culture success, suggesting that these factors significantly affect research outcomes in GC.

Research perspectives
Future research is directed towards improving GCO culture techniques, taking into account the identified influencing factors, and potentially advancing GC research and personalized medicine.

FOOTNOTES

Co-first authors: Kai-Lin Jiang and Xiang-Xiang Wang.

Author contributions: Jiang KL and Ling JH contributed to the conceptualization; Wang XX was involved in the methodology, literature searching, and data extraction; Liu XJ and Chen YQ are responsible for the software; Jia QL and Yang KM contributed to the formal analysis; Jiang KL and Liu XJ wrote the manuscript; Jiang KL contributed to the figure and table; Ling JH participated in the funding acquisition; and all authors have read and agreed to the published version of the manuscript.

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