Received: 6 November 2022 | Revised: 16 March 2023 | Accepted: 17 May 2023

DOI: 10.1002/cac2.12445

CANCER COMMUNICATIONS Open Access

LETTER TO THE EDITOR

Co-operative roles of IL-4Rα and IL-13Rα1 in the progression of ovarian carcinomas and the survival of ovarian carcinoma patients

Dear Editor,

Recently, there has been a rise in interest in the roles of cytokines and cytokine receptors in malignant human tumors. Cytokine receptors, especially type II interleukin-4 receptor (IL-4R), have recently been examined as novel therapeutic targets of human cancers [1–3]. The type II IL-4R complex is composed of IL-4Rα and interleukin-13 receptor α1 (IL-13Rα1), and the expression of IL-4Rα and IL-13Rα1 was higher in cancer tissues compared with normal counterpart tissues [3, 4]. Furthermore, higher expression of IL-4Rα or IL-13Rα1 was associated with shorter survival of cancer patients [5, 6]. This expression of IL-4Rα and IL-13Rα1 in human cancer is related to their roles in regulating signaling mechanisms involved in cancer progression, including the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway [3, 5]. Therefore, the IL-4R-related pathway is targeted in anti-cancer therapeutic strategies [3, 5, 6]. However, despite extensive investigations into the role of IL-4Rα and IL-13Rα1 in the regulation of the biological mechanism of immune cells and tumor cells [2–4], there have been limited reports focused on the role of IL-4Rα/IL-13Rα1 in ovarian carcinomas. Recently, cytokine-related activation of the JAK2/STAT3 pathway was implicated in the progression of ovarian cancers through the induction of epithelial-to-mesenchymal transition (EMT) [7]. In addition, it was shown that EMT is a vital mechanism in the progression of human cancer and resistance of cancers to conventional anti-cancer therapies [8, 9]. Therefore, when considering the roles of IL-4Rα and IL-13Rα1 in JAK-related cancer progression [2, 3, 5], IL-4Rα and IL-13Rα1 might be involved in the progression of ovarian cancers. Therefore, this study investigated the expression of IL-4Rα and IL-13Rα1 in human ovarian cancer tissues and further evaluated the roles of IL-4Rα and IL-13Rα1 in the progression of ovarian carcinomas in conjunction with the EMT phenotype. Detailed study methods are described in the Supplementary Materials and Methods.

In human ovarian carcinomas, IL-4Rα and IL-13Rα1 were primarily expressed in the cytoplasm and nuclei of tumor cells (Figure 1A). Clear expression of IL-4Rα or IL-13Rα1 in the cytoplasmic membrane was not seen. The positivity of immunohistochemical expression of IL-4Rα and IL-13Rα1 was determined with receiver operating characteristic curve analysis (Supplementary Figure S1). IL-4Rα positivity was significantly associated with older age, increased serum level of cancer antigen 125, higher TNM stage, higher histologic grade, and IL-13Rα1 positivity, whereas IL-13Rα1 positivity was significantly associated with higher tumor stage and bilateral tumor (Supplementary Table S2). In univariate survival analysis, IL-4Rαand IL-13Rα1 expression were significantly associated with both overall survival (OS) and relapse-free survival (RFS) (all $P < 0.01$; Figure 1B, Supplementary Table S3). In addition, the expression of IL-4Rα and IL-13Rα1 were significantly associated with OS and RFS in 97 serous carcinomas (Supplementary Figure S2A), but limited significance in low-grade serous carcinomas, high-grade serous carcinomas, endometrioid carcinomas, and mucinous carcinomas (Supplementary Figure S2B-E). In multivariate analysis, IL-13Rα1 positivity (OS, $P = 0.010$) and IL-4Rα positivity (RFS, $P = 0.029$) were independent indicators of patient survival (Supplementary Table S3). Furthermore, the combined expression patterns of IL-4Rα and IL-13Rα1 were significantly associated with the survival of overall ovarian carcinomas (Figure 1C-D, Supplementary Tables S4-S5), serous carcinomas, and mucinous carcinomas (Supplementary Figure S3A-B), but limited significance in low-grade serous carcinomas, high-grade serous carcinomas, and endometrioid carcinomas (Supplementary Figure S3C-E). Multivariate analysis

Abbreviations: IL-4R, interleukin-4 receptor; IL-13Rα1, interleukin-13 receptor α1; EMT, epithelial-to-mesenchymal transition; JAK, Janus kinase; OS, overall survival; RFS, relapse-free survival; STAT, signal transducer and activator of transcription..

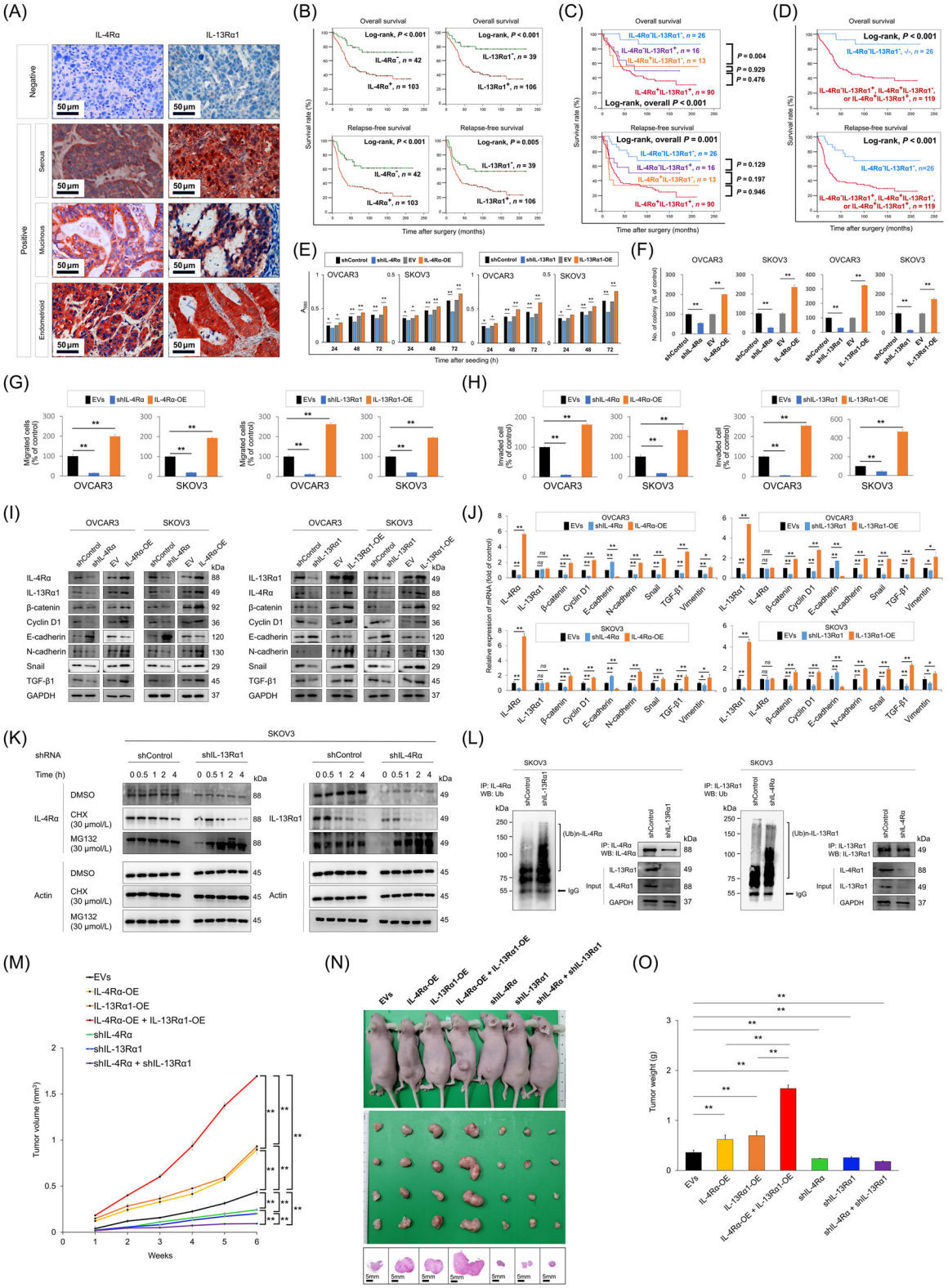

FIGURE 1 The roles of IL-4Rα and IL-13Rα1 in 145 human ovarian carcinomas and ovarian cancer cells. (A) Immunohistochemical expression of IL-4Rα and IL-13Rα1 in serous, mucinous, and endometrioid carcinoma of the ovary. (B) Kaplan-Meier survival curves for overall survival and relapse-free survival according to the expression of IL-4Rα and IL-13Rα1 in 145 ovarian carcinoma patients. (C) Survival analysis according to the co-expression patterns of IL-4Rα and IL-13Rα1 in 145 ovarian carcinomas. Kaplan-Meier survival curves for four subgroups of ovarian carcinomas according to the expression of IL-4Rα and IL-13Rα1: IL-4Rα$^-$IL-13Rα1$^-$, IL-4Rα$^-$IL-13Rα1$^+$, IL-4Rα$^+$IL-13Rα1$^-$, and IL-4Rα$^+$IL-13Rα1$^+$ subgroups. (D) Kaplan-Meier survival curves in two subgroups of ovarian carcinomas: (IL-4Rα$^-$IL-13Rα1$^-$) and (IL-4Rα$^-$IL-13Rα1$^+$, IL-4Rα$^+$IL-13Rα1$^-$, or IL-4Rα$^+$IL-13Rα1$^+$) subgroups. (E, F) CCK8 proliferation assays (E) and

indicated the combined expression patterns of IL-4Rα and IL-13Rα1 to be an independent prognostic indicator of OS ($P = 0.005$) and RFS ($P = 0.025$) (Supplementary Table S5).

Furthermore, in OVCAR3 and SKOV3 cells, knockdown of either IL-4Rα or IL-13Rα1 significantly inhibited the proliferation of cells (Figure 1E-F, Supplementary Figure S4). In contrast, overexpression of either IL-4Rα or IL-13Rα1 significantly increased the proliferation of ovarian cancer cells (Figure 1E-F, Supplementary Figure S4). In addition, the knockdown of either IL-4Rα or IL-13Rα1 significantly decreased the migration and invasion activities of OVCAR3 and SKOV3 cells, but overexpression of either IL-4Rα or IL-13Rα1 significantly increased these activities (Figure 1G-H, Supplementary Figure S5). Furthermore, IL-4Rα- or IL-13Rα-mediated invasiveness of ovarian cancer cells was associated with the expression of the molecules related to EMT. The knockdown of IL-4Rα or IL-13Rα1 increased the expression of E-cadherin, an important indicator of EMT, and decreased the expression of N-cadherin, Snail, transforming growth factor β1 (TGF-β1), β-catenin, and Cyclin D1 (Figure 1I-J, Supplementary Figure S6). In contrast, overexpression of IL-4Rα or IL-13Rα1 decreased the expression of E-cadherin and increased the expression of N-cadherin, Snail, TGF-β1, β-catenin, and Cyclin D1 (Figure 1I-J, Supplementary Figure S6). The knockdown or overexpression of IL-4Rα influenced the expression of IL-13Rα1 protein and vice versa (Figure 1I). However, the overexpression of IL-4Rα or IL-13Rα1 did not influence the expression of mRNA of IL-4Rα or IL-13Rα1 (Figure 1J). Therefore, these data suggest that IL-4Rα and IL-13Rα1 might be involved in the stabilization of each other. As expected, immunoprecipitation of IL-4Rα or IL-13Rα1 showed direct binding of IL-4Rα and IL-13Rα1 proteins (Supplementary Figure S7). In addition, cycloheximide-mediated degradation of IL-4Rα was accelerated with the knockdown of IL-13Rα1 and vice versa (Figure 1K). Furthermore, poly-ubiquitination of IL-4Rα increased with the knockdown of IL-13Rα1, and poly-ubiquitination of IL-13Rα1 increased with the knockdown of IL-4Rα (Figure 1L). This result suggests that both IL-4Rα and IL-13Rα1 are involved in the post-translational stabilization of each other.

Thereafter, we evaluated the co-operative effects of IL-4Rα and IL-13Rα1 in tumor growth after co-transfection (Supplementary Figure S8A). Overexpression of both IL-4Rα and IL-13Rα1 significantly increased proliferation and co-knockdown of IL-4Rα and IL-13Rα1 significantly decreased proliferation of SKOV3 cells in a CCK8 assay and a colony-forming assay (Supplementary Figure S8B-C). In a xenograft animal model, overexpression of either IL-4Rα or IL-13Rα1 significantly increased proliferation compared with controls (Figure 1M-O). Furthermore, tumors overexpressing both IL-4Rα and IL-13Rα1 were significantly larger than tumors overexpressing IL-4Rα or IL-13Rα1 (Figure 1M-O). Knockdown of IL-4Rα, IL-13Rα1, and both IL-4Rα and IL-13Rα1 significantly decreased tumor size compared with controls (Figure 1M-O). Therefore, when considering the results of the expression of IL-4Rα and IL-13Rα1 in human ovarian cancers and ovarian cancer cells, our results suggest that IL-4Rα and IL-13Rα1 are co-operatively involved in the progression of ovarian carcinomas by stabilizing each other. In addition, based on our finding that the knockdown of IL-4Rα and/or IL-13Rα1 inhibits the proliferation and invasiveness of ovarian cancer cells, blocking IL-4, IL-13, IL-4Rα, IL-13Rα1, or molecules downstream of IL-4Rα and IL-13Rα1 signaling might be effective therapeutic targets for ovarian cancers.

In conclusion, this study showed high expression of IL-4Rα and IL-13Rα1 to be an indicator of poor

colony-forming assay (F) after knockdown or overexpression of IL-4Rα and IL-13Rα1 in OVCAR3 and SKOV3 ovarian cancer cells. (G, H) Migration (G) and invasion (H) assays after knockdown or overexpression of IL-4Rα and IL-13Rα1 in OVCAR3 and SKOV3 ovarian carcinoma cells. (I, J) Western blotting (I) and quantitative reverse-transcription polymerase chain reaction (J) after knockdown or overexpression of IL-4Rα and IL-13Rα1 in ovarian carcinoma cells. (K) SKOV3 cells were transfected with the control vector or shRNA for IL-4Rα and IL-13Rα1 and treated with 30 μmol/L cycloheximide or 30 μmol/L MG132 for 0.5 to 4.0 h. Thereafter, the protein lysates were immunoblotted with IL-4Rα, IL-13Rα1, and β-actin. (L) Total protein lysate from SKOV3 cells transfected with empty vector or shRNA for IL-4Rα and IL-13Rα1 and treated with 30 μmol/L MG132 for two h was immunoprecipitated with IL-4Rα and IL-13Rα1 and immunoblotted with anti-ubiquitin and anti-IL-4Rα or anti-IL-13Rα1 antibodies. (M) In vivo evaluation of tumor growth was assessed by subcutaneously implanting 2×10^6 SKOV3 cells that had been transfected with the indicated plasmids. Each week after tumor implantation, the volume of the tumor was measured by the equation of "volume = length × width × height × 0.52" mm^3. (N) Gross and histologic findings of resected tumors. The mice were euthanized at 6 weeks after tumor inoculation, and histologic sections were H&E stained. (O) Weight of resected tumor at 6 weeks after implantation. *$P < 0.05$, **$P < 0.001$. Abbreviations: *ns*, not significant; IL-4Rα, interleukin-4 receptor α; IL-13Rα1, interleukin-13 receptor α1; IL-4Rα$^-$, negative for IL-4Rα; IL-4Rα$^+$, positive for IL-4Rα; IL-13Rα1$^-$, negative for IL-13Rα1; IL-13Rα1$^+$, positive for IL-13Rα1; CHX, cycloheximide; EVs, empty vectors; shRNA, short hairpin RNA; IL-4Rα-OE, vector for wild-type IL-4Rα; IL-13Rα1-OE, vector for wild-type IL-13Rα1; IL-4Rα-OE + IL-13Rα1-OE, vector for wild-type IL-4Rα and wild-type IL-13Rα1; shControl, control vector for shRNA; shIL-4Rα, vector for shRNA for IL-4Rα; shIL-13Rα1, vector for shRNA for IL-13Rα1; shIL-4Rα + shIL-13Rα1, vector for shRNA for IL-4Rα and shRNA for IL-13Rα1; IP, immunoprecipitation; WB, western blotting.

prognosis of ovarian carcinoma patients, and both IL-4Rα and IL-13Rα1 are co-operatively involved in the progression of ovarian carcinomas by stabilizing each other from proteasomal degradation. In addition, blocking IL-4Rα and IL-13Rα1 inhibited proliferation, invasiveness, and the EMT phenotype in ovarian cancer cells. Therefore, this study suggests that a therapeutic modality targeting the IL-4, IL-13, IL-4Rα, or IL-13Rα1 pathway might be a novel therapeutic approach for ovarian carcinoma patients with tumors expressing high levels of IL-4Rα and/or IL-13Rα1.

DECLARATIONS

AUTHOR CONTRIBUTIONS

WKC, UKH, AGA, JZ, KMK, ARA, HSP, SHP, DHC, and KYJ participated in the study design. WKC, UKH, AGA, JZ, KMK, ARA, HSP, SHP, DHC, and KYJ performed the experiment. WKC, HSP, DHC, and KYJ were involved in data collection and data interpretation. WKC, UKH, AGA, JZ, SHP, DHC, and KYJ participated in the statistical analyses. WKC, UKH, AGA, JZ, KMK, ARA, HSP, SHP, DHC, and KYJ wrote the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We thank DB Leveson-Gower for providing medical writing services.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

FUNDING INFORMATION

This work was supported by grants from the Medical Research Center Program (2017R1A5A2015061) through the National Research Foundation (NRF), which is funded by the Korean government, the Ministry of Science, ICT and Future Planning (MSIP).

CONSENT FOR PUBLICATION

Not applicable

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study obtained institutional review board approval from Jeonbuk National University Hospital (IRB number, CUH 2021-04-047-001) and was performed according to the Declaration of Helsinki. Based on the retrospective and anonymous character of the study, the approval contained a waiver for written informed consent. All animal experiments were performed with the approval of the institutional animal care and use committee of Jeonbuk National University (approval number: JBNU 2021-0164).

DATA AVAILABILITY STATEMENT

The datasets used in the current study are available from the corresponding author upon reasonable request.

Won Ku Choi[1,#]
Usama Khamis Hussein[2,3,4,#]
Asmaa Gamal Ahmed[5,#]
Junyue Zhang[6,#]
Kyoung Min Kim[2]
Ae Ri Ahn[2]
Ho Sung Park[2]
See-Hyoung Park[7]
Dong Hyu Cho[1,8,9]
Kyu Yun Jang[2,8,9]

[1]*Department of Obstetrics and Gynecology, Jeonbuk National University Medical School, Jeonju, Republic of Korea*
[2]*Department of Pathology, Jeonbuk National University Medical School, Jeonju, Republic of Korea*
[3]*Faculty of Science, Beni-Suef University, Beni-Suef, Egypt*
[4]*School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, Texas, USA*
[5]*Faculty of Postgraduate Studies and Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt*
[6]*Department of Orthopedic Surgery, Jeonbuk National University Medical School, Jeonju, Republic of Korea*
[7]*Department of Bio and Chemical Engineering, Hongik University, Sejong, Republic of Korea*
[8]*Research Institute of Clinical Medicine of Jeonbuk National University, Jeonju, Republic of Korea*
[9]*Biomedical Research Institute of Jeonbuk National University Hospital, Jeonju, Republic of Korea*

Correspondence
Kyu Yun Jang, Department of Pathology, Jeonbuk National University Medical School, Jeonju 54896, Republic of Korea.
Email: kyjang@jbnu.ac.kr

Dong Hyu Cho, Department of Obstetrics and Gynecology, Jeonbuk National University Medical School, Jeonju 54896, Republic of Korea.
Email: obgyn2001@jbnu.ac.kr

See-Hyoung Park, Department of Bio and Chemical Engineering, Hongik University, Sejong 30016, Republic of Korea.
Email: shpark74@hongik.ac.kr

[#]Won Ku Choi, Usama Khamis Hussein, Asmaa Gamal Ahmed, and Junyue Zhang contributed equally to the study.

ORCID

Kyu Yun Jang https://orcid.org/0000-0002-5276-4446

REFERENCES

1. Bankaitis KV, Fingleton B. Targeting IL4/IL4R for the treatment of epithelial cancer metastasis. Clin Exp Metastasis 2015;32(8):847–56.
2. Hallett MA, Venmar KT, Fingleton B. Cytokine stimulation of epithelial cancer cells: the similar and divergent functions of IL-4 and IL-13. Cancer Res 2012;72(24):6338–43.
3. Suzuki A, Leland P, Joshi BH, Puri RK. Targeting of IL-4 and IL-13 receptors for cancer therapy. Cytokine 2015;75(1):79–88.
4. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, et al. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. Cell 2008;132(2):259–72.
5. Kang MA, Lee J, Ha SH, Lee CM, Kim KM, Jang KY, et al. Interleukin4Ralpha (IL4Ralpha) and IL13Ralpha1 Are Associated with the Progress of Renal Cell Carcinoma through Janus Kinase 2 (JAK2)/Forkhead Box O3 (FOXO3) Pathways. Cancers (Basel) 2019;11(9):1394.
6. Kim KM, Hussein UK, Park SH, Moon YJ, Zhang Z, Ahmed AG, et al. Expression of IL4Ralpha and IL13Ralpha1 are associated with poor prognosis of soft-tissue sarcoma of the extremities, superficial trunk, and retroperitoneum. Diagn Pathol 2021;16(1):2.
7. Maccio A, Madeddu C. Inflammation and ovarian cancer. Cytokine 2012;58(2):133–47.
8. Loret N, Denys H, Tummers P, Berx G. The Role of Epithelial-to-Mesenchymal Plasticity in Ovarian Cancer Progression and Therapy Resistance. Cancers (Basel) 2019;11(6):838.
9. Du B, Shim JS. Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. Molecules 2016;21(7):965.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.