Gynecologic Oncology Reports 50 (2023) 101302

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Therapeutic target biomarkers of patient-derived xenograft models of gastric-type cervical adenocarcinoma

Yuki Kojima [a,b,*], Hiroshi Yoshida [c], Toshihiro Okuya [a], Hitomi S Okuma [a], Tadaaki Nishikawa [a], Maki Tanioka [a], Kazuki Sudo [a], Emi Noguchi [a], Tatsunori Shimoi [a], Kenji Tamura [a], Yasuhito Tanase [d], Masaya Uno [d], Mitsuya Ishikawa [d], Motoko Arakaki [a,e], Hitoshi Ichikawa [e], Shigehiro Yagishita [b], Akinobu Hamada [b], Yasuhiro Fujiwara [a], Kan Yonemori [a], Tomoyasu Kato [d]

[a] Department of Medical Oncology, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan
[b] Department of Molecular Pharmacology, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan
[c] Department of Diagnostic Pathology, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan
[d] Department of Gynecology, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan
[e] Department of Clinical Genomics, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan

ARTICLE INFO

Keywords:
Cervical cancer
Gastric-type adenocarcinoma
Biomarker
Patient-derived xenograft

ABSTRACT

Background: Most cervical adenocarcinomas are associated with human papillomavirus (HPV). Gastric-type cervical adenocarcinoma (GAS), an HPV-independent adenocarcinoma, shows an aggressive clinical feature, resulting in a poor prognosis. Resistance to chemotherapy poses a difficulty in managing patients with metastatic GAS. We aimed to establish patient-derived xenografts (PDXs) of tumors from two patients with GAS and evaluated protein biomarkers for drug development using immunohistochemistry.

Methods: Two PDXs were established 78 and 48 days after transplanting the patient's tumor tissues into immunodeficient mice, respectively. PDX and patient's tumor samples were stained for HER2, HER3, PMS2, MSH6, PanTrk, and ARID1A to evaluate biomarkers for therapeutic targets. In addition, whole exome sequencing and RNA sequencing were performed on available samples.

Results: The pathological findings in morphological features and immunohistochemical profiles from the established PDXs were similar to those from the patients' surgical tumor specimens. HER3 was overexpressed in the patient's tumors, and the corresponding PDX tumors and HER2 was weakly stained in both types of tumor samples. In all PDX and patient tumor samples, PMS2, MSH6, and ARID1A were retained, and PanTrk was not expressed. In addition, a total of 10 samples, including tumor tissue samples from 8 other GAS patients, were evaluated for HER3 expression scores, all of which were 2 + or higher.

Conclusions: In summary, we evaluated biomarkers for therapeutic targets using newly established PDX models of GAS. Frequent HER3 overexpression and HER2 expression in GAS tumors suggest the possibility of new treatments for patients with GAS by targeting HER3 and HER2.

1. Background

Cervical cancer is the fourth most common cancer among women worldwide, accounting for approximately 6.5% of all female patients with cancer (Sung et al., 2021). The most histological type of cervical cancer is squamous cell carcinoma (SCC), with adenocarcinoma (AC) as the second most common, accounteding for 15–25% (Castanon et al., 2016; Galic et al., 2012). AC incidence is increasing relatively, although SCC incidence has decreased due to vaccination and screening in developed countries (Castanon et al., 2016). Most cervical ACs are associated with human papillomavirus (HPV), mainly HPV types 18 and 16. In 2018, an international group of gynecological pathologists proposed a new pathogenetic scheme, the International Endocervical Adenocarcinoma Criteria and Classification (IEACC) (Stolnicu et al., 2018). In the classification, AC is divided into HPV-associated and HPV-independent types (Stolnicu et al., 2018). Since most cervical cancers are associated with HPV infection, The World Health Organization (WHO) has proposed increased vaccination and screening to eradicate

* Corresponding author at: Department of Medical Oncology, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan.
E-mail address: yuukojim@ncc.go.jp (Y. Kojima).

https://doi.org/10.1016/j.gore.2023.101302
Received 26 September 2023; Received in revised form 4 November 2023; Accepted 4 November 2023
Available online 14 November 2023

the disease (Brisson et al., 2020). However, HPV-independent AC is poorly treated and has a poor prognosis.

GAS was added to the WHO classification in 2014 and is characterized by the absence of HPV infection (Mikami et al., 2004; Yoshida et al., 2021). GAS have an aggressive clinical feature, resulting in a poor prognosis compared to HPV-associated ACs. Resistance to chemotherapy poses a difficulty in managing patients with metastatic GAS. It is unclear whether the same treatment strategy applicable to SCC can be adapted to patients with GAS. Therefore, there is a need to develop treatments targeting GAS.

The patient-derived xenograft (PDX) models have attracted attention as a cancer research platform with a high predictive clinical efficacy. Compared to traditional cell line and cell line xenograft models, PDXs preserve tumor heterogeneity and provide a more accurate reflection of the clinical efficacy of treatment (Gao et al., 2015). In the current study, we aimed to establish GAS PDX models based on tumors from two patients and evaluated protein biomarkers for GAS for drug development using immunohistochemical staining.

2. Methods

2.1. Patient specimens and PDX establishment

PDX models were established based on tumors from two patients diagnosed with GAS at the National Cancer Center Hospital, Japan, provided by the National Cancer Center J-PDX Library, Japan (Yagishita et al., 2021). The method of establishing the PDX is briefly described. The surgical samples were used 2 to 10–mm^3. Tissues were immediately soaked in storage solution (Theliokeep, Bio Verde Inc) after collection and stored at 4 °C. The transplantation site was subcutaneous around the flank of female 6-week-old NOG mice (NOD. Cg-Prkdc^{scid} Il2rg^{tm1Sug}/ShiJic, In-Vivo Science Inc). Mice were monitored weekly for tumor growth and body weight. All mice were euthanized by cervical dislocation under anesthesia, and tumors were removed. Patient characteristics are listed in Table 1. Two PDX models were established 78 and 48 days after the transplantation of two patient tumor tissues into immunodeficient mice, respectively (Table 2).

To evaluate the reproducibility of protein expression in PDX specimens, we also evaluated tumor tissue from eight other patients diagnosed with GAS at our institution. The characteristics of these patients are listed in Supplemental Table 1. The histological types of these specimens were defined according to the WHO classification of uterine cervix tumors (4th edition).

The protocol for the human study was reviewed and approved by the ethics committee of the National Cancer Center Hospital, Japan (2014–393 and 2015–123) and was conducted in full concordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. The study design and conduct complied with all applicable regulations, guidelines, and local policies.

Table 1
Patient characteristics.

	Case 1	Case 2
Age, year	38	34
Race	Asian (Japanese)	Asian (Japanese)
Performance status	0	0
Past medical history	none	none
Smoking history	no	no
FIGO Stage (2018)	IIA1	IB2
pT	2b	2a2
Tumor size in greatest dimension	4.8 cm	6.0 cm
Depth of stromal invasion	18 mm thick	18 mm thick
Lymphatic invasion	positive	positive
Vascular Invasion	positive	positive
pN	0	0
Cervix, Dysplasia	absent	absent

FIGO, International Federation of Gynecology and Obstetrics.

Table 2
Summary of the establishment of PDX models.

	Case 1	Case 2
Type of patient sample	Surgery sample	Surgery sample
Disease site of sample	Primary site	Primary site
Prior chemotherapy	None	None
Prior radiotherapy	None	None
Time *in vivo* for serial xenograft, days	78	48

Animal experiments were performed in compliance with the guidelines of the Institute for Laboratory Animal Research, National Cancer Center Research Institute (T17-073 and T19-008).

2.2. Immunohistochemical analysis

Hematoxylin and eosin (HE)-stained tumor tissues on slides from patient tumor tissue and PDX samples were reviewed to obtain representative sections. New 4-μm-thick sections of formalin-fixed paraffin-embedded surgical specimens were prepared and were immunohistochemically stained. Immunohistochemistry staining was performed using the following antibodies: p16 (clone E6H4, Roche diagnostics), MUC5AC (clone CLH2, Novocastra), p53 (clone DO7, Dako), HER2/ErbB2 (Herceptest, Dako), HER3/ErbB3 (clone D22C5, Cell Signaling Technology), PMS2 (clone EP51, Dako), MSH6(clone EP49, Dako), Pan-Trk (clone EPR17341, Abcam), ARID1A (rabbit polyclonal, Sigma), according to the manufacturer's instructions. The tumor tissue sections were counterstained with hematoxylin.

An experienced pathologist evaluated HER2 (also known as *ERBB2*) expression following the HER2 testing guideline for gastroesophageal cancer from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology (Bartley et al., 2016). HER3 (also known as *ERBB3*) expression was assessed similarly for HER2 as previously described (Mizuno et al., 2020). Strong and continuous nuclear and cytoplasmic expression of p16 in all epithelial cells were regarded as block positive staining, which was reported as a good surrogate test for a potentially transforming HPV infection. Aberrant p53 staining was defined as a strong and diffuse nuclear staining pattern (>70% of carcinoma cells) or completely negative ("null pattern") staining of carcinoma cells, with appropriate staining of surrounding non-tumor cells as an internal positive control. A weak and heterogeneous staining pattern of tumor cells was classified as the wild-type pattern. Immunohistochemistry (IHC) staining of PMS2 and MSH6 alone was reported to represent the staining with a four-antibody (comprising MLH1, MSH2, MSH6, and PMS2) panel for dMMR screening (Hall et al., 2010). Therefore, in this study, MMR-deficient status was defined as the complete loss of nuclear staining for PMS2 and/or MSH6 proteins. This is because gene mutation and the loss of MLH1 and MSH2 invariably result in the degradation of PMS2 and MSH6, respectively, due to the binding properties of the mismatch repair heterodimer complexes. Normal mucosa, stromal cells, and inflammatory cells adjacent to tumor cells were used as positive controls for IHC staining. Cytoplasmic and/or nuclear staining of Pan-Trk was assessed. Lymphocytes and endocervical glandular epithelia were used as negative controls. Nuclear expression of ARID1A by IHC was categorized into retained expression (positivity in almost all tumor cells) or any loss of expression. Stainings of adjacent normal epithelial cells were used as positive controls.

2.3. Whole-exome sequencing

The genome DNAs of the PDX and patient tumor samples and a matched germline sample (peripheral blood) were subjected to whole-exome sequencing analysis. Sequencing libraries were constructed using the SureSelect XT Human All Exon V7 capture library (Agilent Technologies, St. Clara, CA, USA), the SureSelect XT Library Prep Kit

(Agilent Technologies), and the KAPA Hyper Prep Kit (KAPA Biosystems, Wilmington, MA, USA); the raw sequence data were generated by the NextSeq 500 sequencer (Illumina, San Diego, CA, USA). The resulting sequence reads were aligned to a combined human (GRCh38 analysis set) and mouse (GRCm38) genome reference using the Burroughs Wheeler Aligner (BWA)-MEM algorithm. Mutations [single-nucleotide variants (SNVs) and short insertions and deletions (Indels)] were detected using Mutect2. Mutations in intergenic, intronic (other than splicing junctions), and untranslated regions were excluded.

2.4. RNA sequencing

The total RNAs extracted from the PDX and the patient's original tumor tissues were subjected to exome RNA sequencing analysis. Sequencing libraries were constructed using the SureSelect XT Human All Exon V7 capture library and the SureSelect XT HS2 RNA Reagent Kit (Agilent Technologies). The raw sequence data were generated by the NextSeq 500 sequencer. Using the STAR algorithm, the resulting sequence reads were aligned to a combined human (GRCh38 primary assembly with GENCODEv37) and mouse (GRCm38 with Ensembl 100) genome reference. The transcripts per million (TPM) value for each gene was calculated using the StringTie algorithm for gene expression analysis.

3. Results

3.1. Pathological findings of PDX models of GAS

HE staining showed abundant, clear to pale eosinophilic cytoplasm with the well-defined cell membrane in patient tumor and PDX samples (Supplementary Fig. 1). In addition, the expression status of the proteins for GAS diagnosis was evaluated (Fig. 1). Tumor samples from both PDX models showed no p16 block positivity, similar to the findings from patient tumor samples. Similarly, tumor samples from both PDX models showed positive staining of MUC5AC and p53.

Immunohistochemistry staining was performed for p16, MUC5AC, and p53. In all tumors examined, p16, used as a surrogate marker for HPV-related tumors, does not exhibit a block-type positive pattern, aligning with the diagnosis of HPV-independent gastric-type adenocarcinoma. (A-D) Immunohistochemical staining of p16 is either completely negative or just focally positive. (E-H) No p16 block positivity is observed. (I-L) The same staining pattern of MUC5AC is seen in the pair of the patient's original tumor tissue and the corresponding PDX tumor tissue. The diffused strong staining pattern of p53 is observed in the pair of the patient's original tumor tissue and the corresponding PDX tumor tissue. All images are shown at the magnification of 200x.

3.2. Evaluation of therapeutic biomarkers according to immunohistochemical staining

HER2, HER3, PMS2, MSH6, PanTrk, and ARID1A were stained for PDX and patient tumor samples to evaluate biomarkers for therapeutic targets. All staining results were consistent between patient tumor samples and the tumor samples from the PDX models derived from both patients.

The immunostaining results for HER3 and HER2 are shown in Fig. 2 and Supplemental Table 2. Staining results for HER3 showed that the scores for both patients' tumors and the corresponding PDX samples were 3+; the staining scores for HER2 were 1 + in both cases. PMS2, MSH6, PanTrk, and ARID1A were also assessed by immunostaining, but no therapeutic target results were obtained (Supplemental Fig. 2).

HER3 and HER2 expression in the patient's original tumor tissues and the PDX tumor tissues was demonstrated at the magnification of 200x. HER3 or HER2 expression was similar in the PDX specimens and the corresponding patient's tumor samples. In all tumors, HER2 expression is equivalent to a score of 1+, and the results for both cases and PDX are concordant. (A-D) HER2 expression score was 1 +. (E-H) HER3 expression score was 3 +.

3.3. Immunohistochemical staining for HER3 and HER2 expression in GAS

Eight additional tumor tissue specimens of patients with GAS diagnosed at our institution were stained for HER3 and HER2. A total of ten pathological specimens of patients with GAS were evaluated, including the specimens from the PDX models (Table 3). HER3 expression scores for all ten samples were 2 + or higher, and the scores for five samples (50%) were 3+ (Supplemental Fig. 3). The HER2 expression score was 3

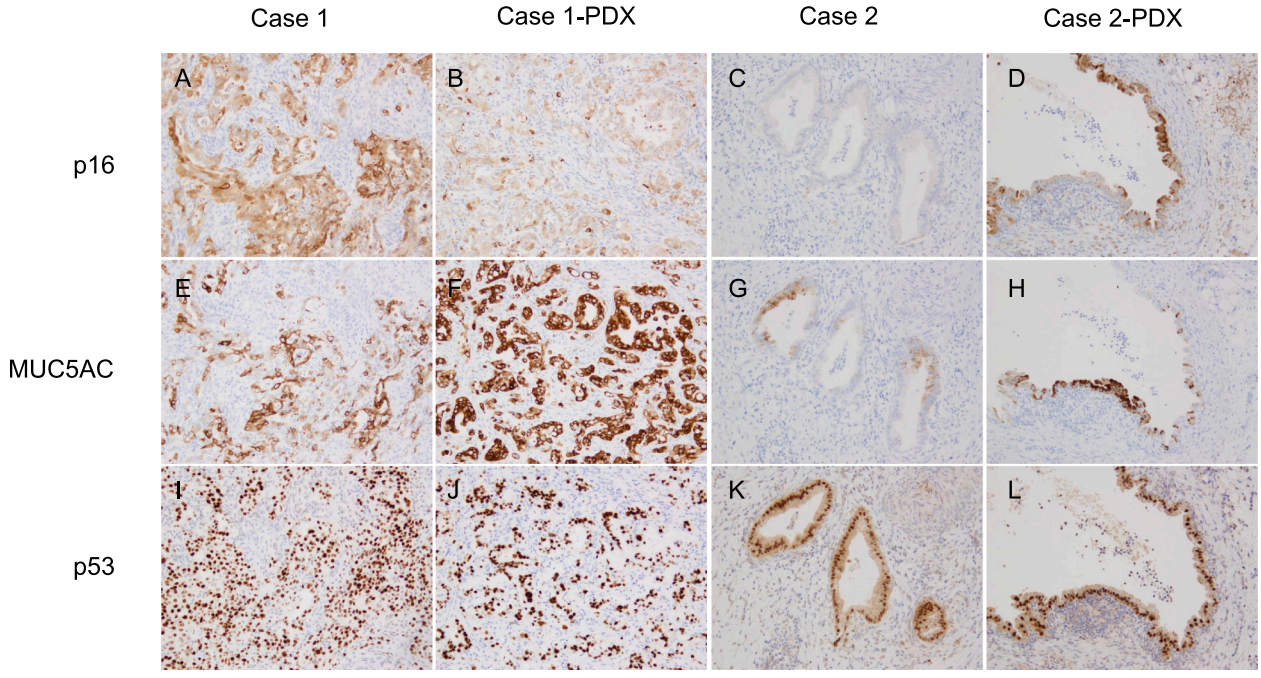

Fig. 1. Shared immunohistochemical profiles between the patient tumor tissues and the corresponding PDX tumor tissues.

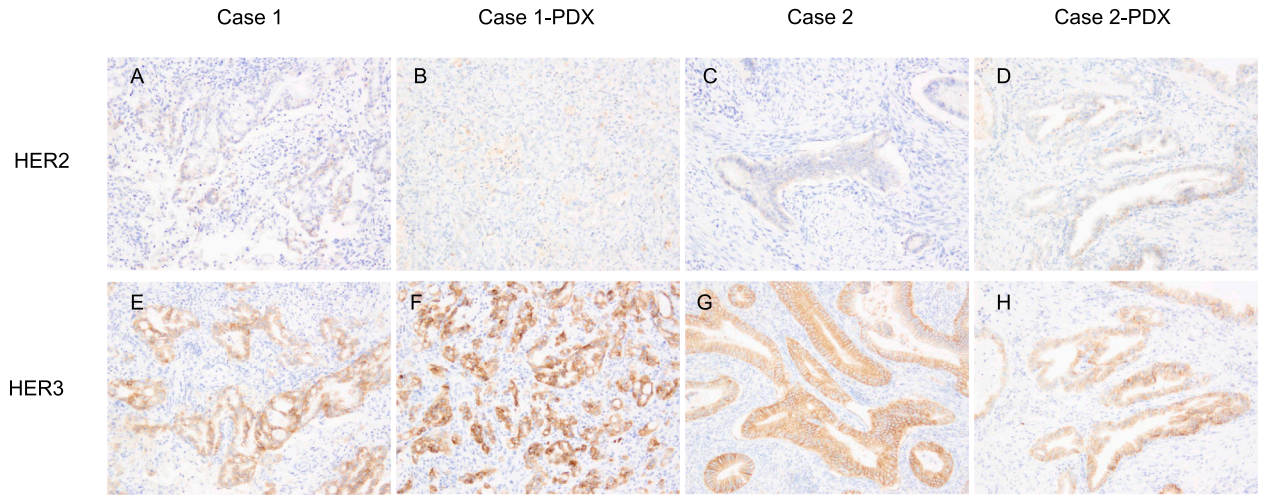

Fig. 2. HER3 and HER2 expression in the parental original tumor tissues and the PDX tumor tissues of gastric-type adenocarcinoma.

Table 3
HER3 and HER2 expression in tissue from the patients with gastric-type adenocarcinoma (N = 10).

#	HER3	HER2
1	2+	1+
2	2+	3+
3	2+	1+
4	2+	1+
5	3+	1+
6	2+	2+
7	3+	2+
8	3+	2+
Case1	3+	1+
Case 2	3+	1+

+ in one, 2 + in three, and 1 + in the remaining six samples.

3.4. Genomic features of a PDX model of GAS

The whole exome sequencing was performed to identify somatic mutations in Case 1 patient tumor and its corresponding PDX tumor samples. Results from these two types of samples exhibited 31 overlapping somatic mutations, including three somatic mutations in *AMER1*, *KRAS*, and *TP53* in Cancer Gene Census genes (Fig. 3A).

Mutation and transcriptomic analyses of a PDX model of gastric-type cervical adenocarcinoma (Case 1). (A) Venn diagram exhibiting the overlap of somatic mutations detected in whole exome sequencing analysis of the patient tumor and the corresponding PDX tumor samples. (B) Transcript abundance (TPM) of the *ERBB2* and *ERBB3* genes was determined by RNA sequencing analysis of the patient tumor and the corresponding PDX tumor samples.

To determine whether gene expression profiles are preserved in the PDX models, we used RNA sequencing to analyze gene expression of patient tumor and PDX samples, and *ERBB2* and *ERBB3* were highly expressed in both types of samples based on IHC staining (Fig. 3B).

4. Discussion

In the current study, we evaluated the expression of therapeutic target proteins using established GAS-PDX models. To our knowledge, this is the first report of PDXs of GAS. HER2, HER3, PMS2, MSH6, PanTrk, and ARID1A were evaluated, and HER3 expression was high. In addition, there was a high frequency of strong HER3 expression in patient samples.

GAS is a clinically aggressive cervical cancer with a distinctive histologic feature. GAS is unrelated to HPV and has poorer outcomes than the usual-type HPV-dependent adenocarcinomas (Kojima et al., 2007; Kusanagi et al., 2010); GAS's unrelatedness to HPV is considered an important feature associated with its aggressiveness. In Europe, GAS incidence was rare, accounting for 1.5% of ACs (Holl et al., 2015); In contrast, it is common in Japan, accounting for up to 20% to 25% of all endocervical ACs (Kojima et al., 2007; Kusanagi et al., 2010). Standard chemotherapies have been performed in patients with recurrent or metastatic cervical cancer; however, the response rates to single and combination therapy remain poor (Tewari et al., 2014). Among cervical cancers, GAS is particularly refractory to anticancer drugs (Kojima et al.,

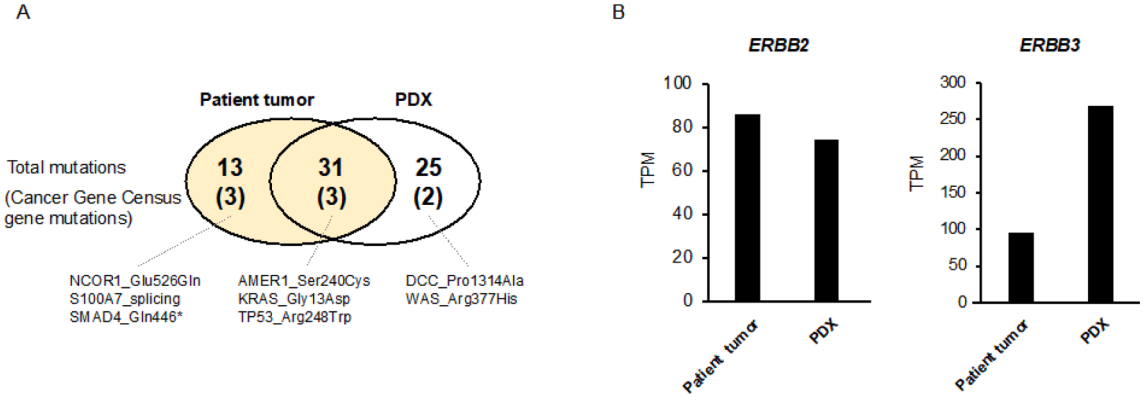

Fig. 3. Genomic profile of a PDX model of gastric-type cervical adenocarcinoma.

2018). GAS has been reported to be difficult to diagnose in its early stages; it is chemoresistant (Kojima et al., 2018) and radioresistant (Nishio et al., 2019) with unknown mechanisms. Notably, GAS is expected to persist in the post-HPV vaccination era. Therefore, there is a need to develop GAS treatments.

The PDX models were established by the direct engraftment of tumor tissue from a patient into an immunocompromised mouse, maintaining the tumor growth *in vivo*. PDX models maintained the characteristics of the parental tumors, such as protein and gene expression profiles. Therefore, they have become important tools for preclinical studies, particularly for investigating cancer pathology and developing new drugs. Recently, the National Cancer Institute decided to replace a panel of 60 human cell lines with PDX models for drug screening (Ledford, 2016). There are several reports on PDX models of cervical cancer, mainly of squamous cell carcinoma (Boone et al., 2015; Dou et al., 2014). GAS is a rare type of cervical cancer, and no previous reports of its PDX models exist. Preclinical data using PDX models may lead to developing new therapies for GAS.

HER3 is overexpressed in several cancer types and is correlated with poor disease prognosis (Ocana et al., 2013; Tanner et al., 2006). For cervical cancer, the incidence of *ERBB3* alterations was higher in patients with adenocarcinoma than in those with squamous cell carcinoma (Cancer Genome Atlas Research et al., 2017). A previous study reported that HER3 expression was associated with poor disease-free survival in early-stage adenocarcinoma and adenosquamous carcinoma of the cervix (Mizuno et al., 2020). HER3 promotes tumor initiation and progression to activate oncogenic signaling via the PI3K/AKT pathway, a major cause of drug resistance (Engelman et al., 2005). Several therapies targeting HER3 have been recently developed, including patritumab deruxtecan, duligotuzumab, and seribantumab (Jaiswal et al., 2013; Janne et al., 2019; Liu et al., 2016) and high expression of HER3 protein is associated with the patient's response to therapies targeting HER3 (Koganemaru et al., 2019). In this study, GAS frequently showed HER3 overexpression; therefore, our findings may provide insight into providing new therapeutic strategies for treating patients with GAS.

HER2 expression in cervical cancer, mainly of squamous cell carcinoma, is 1–21% (Yan et al., 2014). In AC of the cervix, HER2 expression is 11–30% (Halle et al., 2017; Kihana et al., 1994; Shi et al., 2021). Several studies reported that HER2 is expressed in 7.7 to 14.7% of GAS (Nakamura et al., 2019; Shi et al., 2021), similar to previous reports. Anti-HER2 agents are the standard treatment for HER2-positive gastric cancer; and their frequency is about 20%. Similar to gastric cancer, a small fraction of cervical AC and GAS may be effectively treated by anti-HER2 drugs. The clinical significance of HER2-targeting antibody-drug conjugate for low-HER2 expression (Modi et al., 2020) suggests that HER2 expression may be an important therapeutic target.

Several studies investigated the genomic profiles of GAS (Garg et al., 2019; Hodgson et al., 2020; Lu et al., 2021; Park et al., 2021), and we compared the previously discovered mutated genes with those disclosed in this report; we found consistency in the expression of mutated genes, such as *TP53* and *KRAS*, which may be the typical driver mutations in GAS. The genes involved in the PI3K-AKT signaling pathway, frequently found in HPV-associated cervical cancer (Cancer Genome Atlas Research et al., 2017), were not detected in GAS. PDX samples with genetic profiles that include driver mutations are highly significant for developing therapeutic drugs against diseases (Sun et al., 2021). We plan a future *in vivo* study to evaluate drug efficacy using the GAS-PDX models.

5. Conclusions

The current study evaluated protein biomarkers for drug development using newly established PDX models of GAS. Frequent HER3 overexpression and HER2 expression in GAS samples suggest the possibility of therapeutic targeting of HER3 and HER2 for treating patients with GAS.

Ethics approval and consent to participate

The present study was approved by the Ethics committee of the National Cancer Center Hospital (Tokyo, Japan) and National Cancer Center (Tokyo, Japan) (approval numbers: 2015–123), and was conducted in full concordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients. The study design and conduct complied with all applicable regulations, guidelines, and local policies. Animal experiments were performed in compliance with the guidelines of the Institute for Laboratory Animal Research, National Cancer Center Research Institute (T17-073 and T19-008).

Consent for publication

Not applicable.

Availability of data and materials

Sequence files are available in the NBDC database (J-DS000883, URL: https://humandbs.biosciencedbc.jp). Other datasets used in the present study are available from the corresponding author upon reasonable request.

Funding

The National Cancer Center Biobank is supported by the National Cancer Center Research and Development Fund, Japan. National Cancer Center J-PDX library is partly supported by the AMED under Grant Number 17pc0101011h0001 and the National Cancer Center Research and Development Fund, Japan. This work was supported by JSPS KAKENHI (Grant Number: JP20K16430).

CRediT authorship contribution statement

Yuki Kojima: Methodology, Project administration, Funding acquisition, Writing – original draft. Hiroshi Yoshida: Methodology, Formal analysis, Writing – review & editing. **Toshihiro Okuya:** Writing – review & editing. **Hitomi S Okuma:** Writing – review & editing. **Tadaaki Nishikawa:** Writing – review & editing. **Maki Tanioka:** Writing – review & editing. **Kazuki Sudo:** Writing – review & editing. **Emi Noguchi:** Writing – review & editing. **Tatsunori Shimoi:** Writing – review & editing. **Kenji Tamura:** Writing – review & editing. **Yasuhito Tanase:** Writing – review & editing. **Masaya Uno:** Writing – review & editing. **Mitsuya Ishikawa:** Writing – review & editing. **Motoko Arakaki:** Writing – review & editing. **Hitoshi Ichikawa:** Formal analysis, Methodology, Writing – review & editing. **Shigehiro Yagishita:** Writing – review & editing. **Akinobu Hamada:** Funding acquisition, Supervision, Writing – review & editing. **Yasuhiro Fujiwara:** Writing – review & editing. **Kan Yonemori:** Writing – review & editing. **Tomoyasu Kato:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yuki Kojima, Hiroshi Yoshida, Toshihiro Okuya, Hitomi S. Okuma, Kazuki Sudo, Maki Tanioka, Tatsunori Shimoi, Kenji Tamura, Yasuhito Tanase, Masaya Uno, Mitsuya Ishikawa, Motoko Arakaki, Shigehiro Yagishita and Tomoyasu Kato have no conflict interest to disclose; Tadaaki Nishikawa received research funds from Takeda Pharmaceutical Company, Eisai, AstraZeneca, outside the submitted work; Emi Noguchi received research funds from Pfizer, Taiho, Eli Lilly, AstraZeneca, Chugai, Eisai, outside the submitted work; Hitoshi Ichikawa received research funds from Chugai Pharma, Eisai, Healios, Ono Pharmaceutical, outside the submitted work; Akinobu Hamada received

research funds from Shimadzu Corporation, Daiichi Sankyo Company, Chugai Pharmaceutical, AstraZeneca, outside the submitted work; Yasuhiro Fujiwara received research funds from AstraZeneca, Chugai, Daiichi Sankyo, Bristol-Myers, SRL, Santen, outside the submitted work; Kan Yonemori received research funds from Pfizer, AstraZeneca, Eisai, Takeda Pharmaceutical Company, Chugai, Ono Pharmaceutical Company, Novartis, Daiichi Sankyo, outside the submitted work.

Acknowledgments

We thank all patients for participating in this study. We thank Nao Nakamura and Kyoko Onozawa for their secretarial assistance. Editage, Cactus Communications provided editorial support through medical writing, assembling tables and creating high-resolution images based on authors' detailed directions, collating author comments, copyediting, fact-checking, and referencing.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2023.101302.

References

Bartley, A.N., Washington, M.K., Ventura, C.B., Ismaila, N., Colasacco, C., Benson, A.B., Carrato, A., Gulley, M.L., Jain, D., Kakar, S., Mackay, H.J., Streutker, C., Tang, L., Troxell, M., Ajani, J.A., 2016. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. Arch. Pathol. Lab Med. 140 (12), 1345–1363.

Boone, J.D., Dobbin, Z.C., Straughn Jr., J.M., Buchsbaum, D.J., 2015. Ovarian and cervical cancer patient derived xenografts: The past, present, and future. Gynecol. Oncol. 138, 486–491. https://doi.org/10.1016/j.ygyno.2015.05.022.

Brisson, M., Kim, J.J., Canfell, K., Drolet, M., Gingras, G., Burger, E.A., Martin, D., Simms, K.T., Bénard, É., Boily, M.-C., Sy, S., Regan, C., Keane, A., Caruana, M., Nguyen, D.T.N., Smith, M.A., Laprise, J.-F., Jit, M., Alary, M., Bray, F., Fidarova, E., Elsheikh, F., Bloem, P.J.N., Broutet, N., Hutubessy, R., 2020. Impact of HPV vaccination and cervical screening on cervical cancer elimination: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet 395 (10224), 575–590.

Cancer Genome Atlas Research, N., Albert Einstein College of, M., Analytical Biological, S., Barretos Cancer, H., Baylor College of, M., Beckman Research Institute of City of, H., et al., 2017. Integrated genomic and molecular characterization of cervical cancer. Nature. 543, 378-384. https://doi.org/10.1038/nature21386.

Castanon, A., Landy, R., Sasieni, P.D., 2016. Is cervical screening preventing adenocarcinoma and adenosquamous carcinoma of the cervix? Int. J. Cancer 139, 1040–1045. https://doi.org/10.1002/ijc.30152.

Dou, Y.N., Zheng, J., Foltz, W.D., Weersink, R., Chaudary, N., Jaffray, D.A., Allen, C., 2014. Heat-activated thermosensitive liposomal cisplatin (HTLC) results in effective growth delay of cervical carcinoma in mice. J. Control. Release 178, 69–78.

Engelman, J.A., Jänne, P.A., Mermel, C., Pearlberg, J., Mukohara, T., Fleet, C., Cichowski, K., Johnson, B.E., Cantley, L.C., 2005. ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. Proc. Natl. Acad. Sci. USA 102 (10), 3788–3793.

Galic, V., Herzog, T.J., Lewin, S.N., Neugut, A.I., Burke, W.M., Lu, Y.-S., Hershman, D.L., Wright, J.D., 2012. Prognostic significance of adenocarcinoma histology in women with cervical cancer. Gynecol. Oncol. 125 (2), 287–291.

Gao, H., Korn, J.M., Ferretti, S., Monahan, J.E., Wang, Y., Singh, M., Zhang, C., Schnell, C., Yang, G., Zhang, Y., Balbin, O.A., Barbe, S., Cai, H., Casey, F., Chatterjee, S., Chiang, D.Y., Chuai, S., Cogan, S.M., Collins, S.D., Dammassa, E., Ebel, N., Embry, M., Green, J., Kauffmann, A., Kowal, C., Leary, R.J., Lehar, J., Liang, Y., Loo, A., Lorenzana, E., Robert McDonald, E., McLaughlin, M.E., Merkin, J., Meyer, R., Naylor, T.L., Patawaran, M., Reddy, A., Röelli, C., Ruddy, D.A., Salangsang, F., Santacroce, F., Singh, A.P., Tang, Y., Tinetto, W., Tobler, S., Velazquez, R., Venkatesan, K., Von Arx, F., Wang, H.Q., Wang, Z., Wiesmann, M., Wyss, D., Xu, F., Bitter, H., Atadja, P., Lees, E., Hofmann, F., Li, E.n., Keen, N., Cozens, R., Jensen, M.R., Pryer, N.K., Williams, J.A., Sellers, W.R., 2015. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat. Med. 21 (11), 1318–1325.

Garg, S., Nagaria, T.S., Clarke, B., Freedman, O., Khan, Z., Schwock, J., Bernardini, M.Q., Oza, A.M., Han, K., Smith, A.C., Stockley, T.L., Rouzbahman, M., 2019. Molecular characterization of gastric-type endocervical adenocarcinoma using next-generation sequencing. Mod. Pathol. 32 (12), 1823–1833.

Hall, G., Clarkson, A., Shi, A., Langford, E., Leung, H., Eckstein, R.P., Gill, A.J., 2010. Immunohistochemistry for PMS2 and MSH6 alone can replace a four antibody panel for mismatch repair deficiency screening in colorectal adenocarcinoma. Pathology 42 (5), 409–413.

Halle, M.K., Ojesina, A.I., Engerud, H., Woie, K., Tangen, I.L., Holst, F., Høivik, E., Kusonmano, K., Haldorsen, I.S., Vintermyr, O.K., Trovik, J., Bertelsen, B.I., Salvesen, H.B., Krakstad, C., 2017. Clinicopathologic and molecular markers in cervical carcinoma: a prospective cohort study. Am. J. Obstet. Gynecol. 217 (4), 432.e1–432.e17.

Hodgson, A., Howitt, B.E., Park, K.J., Lindeman, N., Nucci, M.R., Parra-Herran, C., 2020. Genomic Characterization of HPV-related and Gastric-type Endocervical Adenocarcinoma: Correlation With Subtype and Clinical Behavior. Int. J. Gynecol. Pathol. 39, 578–586. https://doi.org/10.1097/PGP.0000000000000665.

Holl, K., Nowakowski, A.M., Powell, N., McCluggage, W.G., Pirog, E.C., Collas De Souza, S., et al., 2015. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study. Int. J. Cancer 137, 2858–2868. https://doi.org/10.1002/ijc.29651.

Jaiswal, B., Kljavin, N., Stawiski, E., Chan, E., Parikh, C., Durinck, S., Chaudhuri, S., Pujara, K., Guillory, J., Edgar, K., Janakiraman, V., Scholz, R.-P., Bowman, K., Lorenzo, M., Li, H., Wu, J., Yuan, W., Peters, B., Kan, Z., Stinson, J., Mak, M., Modrusan, Z., Eigenbrot, C., Firestein, R., Stern, H., Rajalingam, K., Schaefer, G., Merchant, M., Sliwkowski, M., de Sauvage, F., Seshagiri, S., 2013. Oncogenic ERBB3 mutations in human cancers. Cancer Cell 23 (5), 603–617.

Janne, P.A., Yu, H.A., Johnson, M.L., Steuer, C.E., Vigliotti, M., Iacobucci, C., Chen, S., Yu, C., Sellami, D.B., 2019. Safety and preliminary antitumor activity of U3–1402: A HER3-targeted antibody drug conjugate in EGFR TKI-resistant, EGFRm NSCLC. J. Clin. Oncol. 37 (15_suppl), 9010.

Kihana, T., Tsuda, H., Teshima, S., Nomoto, K., Tsugane, S., Sonoda, T., et al., 1994. Prognostic significance of the overexpression of c-erbB-2 protein in adenocarcinoma of the uterine cervix. Cancer 73, 148–153. https://doi.org/10.1002/1097-0142(19940101)73:1<148::aid-cncr2820730125>3.0.co;2-c.

Koganemaru, S., Kuboki, Y., Koga, Y., Kojima, T., Yamauchi, M., Maeda, N., et al., 2019. U3-1402, a Novel HER3-Targeting Antibody-Drug Conjugate, for the Treatment of Colorectal Cancer. Mol. Cancer Ther. 18, 2043-2050. https://doi.org/10.1158/1535-7163.MCT-19-0452.

Kojima, A., Mikami, Y., Sudo, T., Yamaguchi, S., Kusanagi, Y., Ito, M., et al., 2007. Gastric morphology and immunophenotype predict poor outcome in mucinous adenocarcinoma of the uterine cervix. Am. J. Surg. Pathol. 31, 664–672. https://doi.org/10.1097/01.pas.0000213434.91868.b0.

Kojima, A., Shimada, M., Mikami, Y., Nagao, S., Takeshima, N., Sugiyama, T., Teramoto, N., Kiyokawa, T., Kigawa, J., Nishimura, R., 2018. Chemoresistance of Gastric-Type Mucinous Carcinoma of the Uterine Cervix: A Study of the Sankai Gynecology Study Group. Int. J. Gynecol. Cancer 28 (1), 99–106.

Kusanagi, Y., Kojima, A., Mikami, Y., Kiyokawa, T., Sudo, T., Yamaguchi, S., Nishimura, R., 2010. Absence of high-risk human papillomavirus (HPV) detection in endocervical adenocarcinoma with gastric morphology and phenotype. Am. J. Pathol. 177 (5), 2169–2175.

Ledford, H., 2016. US cancer institute to overhaul tumour cell lines. Nature 530 (7591), 391.

Liu, J.F., Ray-Coquard, I., Selle, F., Poveda, A.M., Cibula, D., Hirte, H., Hilpert, F., Raspagliesi, F., Gladieff, L., Harter, P., Siena, S., del Campo, J.M., Tabah-Fisch, I., Pearlberg, J., Moyo, V., Riahi, K., Nering, R., Kubasek, W., Adiwijaya, B., Czibere, A., Naumann, R.W., Coleman, R.L., Vergote, I., MacBeath, G., Pujade-Lauraine, E., 2016. Randomized Phase II Trial of Seribantumab in Combination With Paclitaxel in Patients With Advanced Platinum-Resistant or -Refractory Ovarian Cancer. J. Clin. Oncol. 34 (36), 4345–4353.

Lu, S., Shi, J., Zhang, X., Kong, F., Liu, L., Dong, X., Wang, K., Shen, D., 2021. Comprehensive genomic profiling and prognostic analysis of cervical gastric-type mucinous adenocarcinoma. Virchows Arch. 479 (5), 893–903.

Mikami, Y., Kiyokawa, T., Hata, S., Fujiwara, K., Moriya, T., Sasano, H., Manabe, T., Akahira, J.-I., Ito, K., Tase, T., Yaegashi, N., Sato, I., Tateno, H., Naganuma, H., 2004. Gastrointestinal immunophenotype in adenocarcinomas of the uterine cervix and related glandular lesions: a possible link between lobular endocervical glandular hyperplasia/pyloric gland metaplasia and 'adenoma malignum'. Mod. Pathol. 17 (8), 962–972.

Mizuno, T., Kojima, Y., Yonemori, K., Yoshida, H., Sugiura, Y., Ohtake, Y., Okuma, H., Nishikawa, T., Tanioka, M., Sudo, K., Shimomura, A., Noguchi, E., Kato, T., Shimoi, T., Uno, M., Ishikawa, M., Fujiwara, Y., Ohe, Y., Tamura, K., 2020. HER3 protein expression as a risk factor for post-operative recurrence in patients with early-stage adenocarcinoma and adenosquamous carcinoma of the cervix. Oncol. Lett. https://doi.org/10.3892/ol.2020.11899.

Modi, S., Park, H., Murthy, R.K., Iwata, H., Tamura, K., Tsurutani, J., Moreno-Aspitia, A., Doi, T., Sagara, Y., Redfern, C., Krop, I.E., Lee, C., Fujisaki, Y., Sugihara, M., Zhang, L., Shahidi, J., Takahashi, S., 2020. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. J. Clin. Oncol. 38 (17), 1887–1896.

Nakamura, A., Yamaguchi, K., Minamiguchi, S., Murakami, R., Abiko, K., Hamanishi, J., Kondoh, E., Baba, T., Mandai, M., Matsumura, N., 2019. Mucinous adenocarcinoma, gastric type of the uterine cervix: clinical features and HER2 amplification. Med. Mol. Morphol. 52 (1), 52–59.

Nishio, S., Mikami, Y., Tokunaga, H., Yaegashi, N., Satoh, T., Saito, M., Okamoto, A., Kasamatsu, T., Miyamoto, T., Shiozawa, T., Yoshioka, Y., Mandai, M., Kojima, A., Takehara, K., Kaneki, E., Kobayashi, H., Kaku, T., Ushijima, K., Kamura, T., 2019. Analysis of gastric-type mucinous carcinoma of the uterine cervix - An aggressive tumor with a poor prognosis: A multi-institutional study. Gynecol. Oncol. 153 (1), 13–19.

Ocana, A., Vera-Badillo, F., Seruga, B., Templeton, A., Pandiella, A., Amir, E., 2013. HER3 overexpression and survival in solid tumors: a meta-analysis. J. Natl Cancer Inst. 105, 266–273. https://doi.org/10.1093/jnci/djs501.

Park, E., Kim, S.W., Kim, S., Kim, H.-S., Lee, J.-Y., Kim, Y.T., Cho, N.H., 2021. Genetic characteristics of gastric-type mucinous carcinoma of the uterine cervix. Mod. Pathol. 34 (3), 637–646.

Shi, H., Shao, Y., Lu, W., Lu, B., 2021. An analysis of HER2 amplification in cervical adenocarcinoma: correlation with clinical outcomes and the International Endocervical Adenocarcinoma Criteria and Classification. J. Pathol. Clin. Res. 7, 86–95. https://doi.org/10.1002/cjp2.184.

Stolnicu, S., Barsan, I., Hoang, L., Patel, P., Terinte, C., Pesci, A., Aviel-Ronen, S., Kiyokawa, T., Alvarado-Cabrero, I., Pike, M.C., Oliva, E., Park, K.J., Soslow, R.A., 2018. International Endocervical Adenocarcinoma Criteria and Classification (IECC): A New Pathogenetic Classification for Invasive Adenocarcinomas of the Endocervix. Am. J. Surg. Pathol. 42 (2), 214–226.

Sun, H., Cao, S., Mashl, R.J., Mo, C.-K., Zaccaria, S., Wendl, M.C., et al., 2021. Comprehensive characterization of 536 patient-derived xenograft models prioritizes candidatesfor targeted treatment. Nat. Commun. 12 (1) https://doi.org/10.1038/s41467-021-25177-3.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 71 (3), 209–249.

Tanner, B., Hasenclever, D., Stern, K., Schormann, W., Bezler, M., Hermes, M., Brulport, M., Bauer, A., Schiffer, I.B., Gebhard, S., Schmidt, M., Steiner, E., Sehouli, J., Edelmann, J., Läuter, J., Lessig, R., Krishnamurthi, K., Ullrich, A., Hengstler, J.G., 2006. ErbB-3 predicts survival in ovarian cancer. J. Clin. Oncol. 24 (26), 4317–4323.

Tewari, K.S., Sill, M.W., Long, H.J., Penson, R.T., Huang, H., Ramondetta, L.M., Landrum, L.M., Oaknin, A., Reid, T.J., Leitao, M.M., Michael, H.E., Monk, B.J., 2014. Improved survival with bevacizumab in advanced cervical cancer. N. Engl. J. Med. 370 (8), 734–743.

Yagishita, S., Kato, K., Takahashi, M., Imai, T., Yatabe, Y., Kuwata, T., Suzuki, M., Ochiai, A., Ohtsu, A., Shimada, K., Nishida, T., Hamada, A., Mano, H., 2021. Characterization of the large-scale Japanese patient-derived xenograft (J-PDX) library. Cancer Sci. 112 (6), 2454–2466.

Yan, M., Parker, B.A., Schwab, R., Kurzrock, R., 2014. HER2 aberrations in cancer: implications for therapy. Cancer Treat. Rev. 40, 770–780. https://doi.org/10.1016/j.ctrv.2014.02.008.

Yoshida, H., Shiraishi, K., Kato, T., 2021. Molecular Pathology of Human Papilloma Virus-Negative Cervical Cancers. Cancers (Basel). 13 (24), 6351.