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Proteomic approach to identify biomarkers for invasive cervix cancer - a prospective pilot study

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Summary

Purpose of Investigation: Cervical cancer (CC) is the second most common cancer of women worldwide and a leading cause of mortality. Unfortunately, this disease has no current viable serum biomarkers capable of diagnosing CC or predicting prognosis. The present authors' objective was to examine novel serum biomarkers capable of identifying patients with invasive CC and determine their utility in monitoring disease status. *Materials and Methods:* In this IRB approved prospective study, luminex bead array was used to measure 18 different serum protein concentrations in CC patients (n=23), women with precancerous lesions (CIN2-3) (n=20) and patients with normal cervical cytology (n=20). CC patients had blood samples drawn within 30 days of diagnosis and repeat samples post-completion of therapy. MMP7 expression was confirmed by immunohistochemistry (IHC) and scored independently by two pathologists. *Results:* Multiple proteins had different levels in CC, controls, and CIN ($p < 0.05$). MMP7 was most promising for identifying invasive cancer (sensitivity of 88.9%, specificity 95%, $p < 0.001$). IHC confirmed MMP7 expression in invasive CC. MMP7 serum levels were an indicator for progression-free survival (PFS) (HR 3.82, CI: 1.10–13.29, $p = 0.025$) and overall survival (OS) (HR 7.96, CI: 0.91–69.32, $p = 0.03$). *Conclusion:* MMP7 serum concentration was altered when comparing CC, controls, and CIN2-3, and shows potential as being a future biomarker for both identifying invasive CC and patient prognosis. Based on this study, MMP7 warrants further investigation as a biomarker of invasive CC.

Key words: Cervical cancer; Serum proteomics; Biomarkers.

Introduction

Cervical cancer (CC) is the second most common cancer among women in the world and claims over 250,000 lives worldwide per year [1]. The five-year survival in developing countries is less than 50%, whereas in industrialized countries the five-year survival rate is greater than 66%. However, for all women with advanced stage CC, the outlook is grim, with the median survival only being 13 months [2, 3]. Fortunately, screening for CC greatly diminishes the incidence of invasive CC, which has led to a decreased incidence in the developed world. Unfortunately, due to cost, lack of infrastructure, and lack of access to care in developing countries, the incidence of CC remains high [1, 4, 5].

The pathogenesis of CC also contributes to its large incidence worldwide and overall mortality. Most squamous cell CCs are associated with human papillomavirus infection (HPV) infection. The HPV virus uses a variety of viral protein mediators to cause dysregulation of the retinoblastoma protein and p53, resulting in carcinogenesis [6]. This has led to the development of effective, safe vaccines against the HPV viruses that most commonly cause cancer [7, 8]. Despite these developments, CC is still a problem in the United States, with approximately 12,000 cases being diagnosed annually, and almost 4,200 deaths per year [1, 4].

Despite the success of screening and preventative vaccines, the ability to determine CC prognosis and recurrence of a patient by a minimally invasive serum blood test remains elusive. A simple blood test may also provide a reliable method to help screen and monitor patients, especially in under-served areas. The most reliable serum marker for CC at this point is the squamous cell carcinoma antigen (SCCA). There is much debate over the usefulness of SCCA in the literature; it has been reported that it is elevated in 28-88% of patients with squamous cell CC and has little value as a prognostic marker [9-11]. In this study, the authors' objective was to discover novel serum biomarkers' capable of aiding in diagnosis and predicting disease status in patients with CC.

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Materials and Methods

This was a single-institution, prospective observational study examining serial serum samples in patients with invasive squamous cell (n=20) and adenocarcinoma (n=3) CC, pre-invasive cervical intraepithelial neoplasia (CIN II/III), and controls. Controls were patients who had no history of any neoplasm or precancerous condition. CIN I was excluded. This study was approved by the institutional review board at the Medical College of Georgia at Augusta University; a written informed consent was obtained from all subjects or from a legally authorized representative. The ethics committee at Augusta University approved of the consenting procedure used in this study.

Serum samples were obtained at diagnosis and then at subsequent follow up visits. A diagnosis blood sample was defined as a blood sample within 30 days of the diagnosis date and prior to the start of chemotherapy, radiation, or surgery. No evidence of disease (NED) and recurrence were defined in accordance to Revised RECIST criteria (version 1.1) in combination with physical exam and clinical imaging [12]. Patient records were reviewed for clinical data including demographics, treatment, chemotherapy response, and disease status.

Using this data, differences in serum protein concentration were calculated comparing controls to CINs, and to CC patients separated into groups diagnosis, NED, and progression. Both overall survival (OS) and progression-free survival (PFS) were calculated for each clinical subgroup (diagnosis, NED, progression) based on serum protein concentration of each respective protein. The differences in mean protein concentration were also calculated for each disease status and compared across each protein respectively. The blood sample used for analysis of each clinical subgroup was the first sample taken within 30 days of the patient being diagnosed, in remission, or having progressed.

Each blood sample was examined for the serum concentration of 18 different proteins. These proteins were chosen based on literature support for their involvement in CC, as well as being established serum markers for other cancers. These included members of the insulin-like growth factor binding protein family (IGFBP), matrix metalloproteinase (MMP) family, and proteins related to angiogenesis such as VEGF [13-15].

Serum protein levels were measured using bead array kits according to manufacturer's protocol. The kit is based on sandwich immunoassay, which consists of dyed microspheres conjugated with a specific monoclonal capture antibody. Briefly, properly diluted serum samples were incubated overnight for 18 hours at 4°C with the antibody-coupled microspheres and then with biotinylated detection antibody before the addition of streptavidin-phycoerythrin. The captured bead-complexes were measured with array reader using the following instrument settings: events/bead: 50, minimum events: 0, flow rate: 60 µL/min, sample size: 50 µL, and discriminator gate: 8000-13500. Median fluorescence intensity (MFI) was collected and used for calculating protein concentration. If a serum protein concentration was not measurable, it was not included in the data analysis. This specifically included one patient who had no detectable levels of OPN at disease progression.

All statistical analyses were performed using the R language and environment for statistical computing (R version 3.2.2; R Foundation for Statistical Computing; www.r-project.org). The normalized protein concentrations were log2 transformed prior to all statistical analyses to achieve normal distribution. The comparisons between group means were made by ANOVA (for three groups) followed by pair-wise comparisons using Bonferroni post hoc testing. The statistical significance of differences was set at $p < 0.05$. The area-under-the-curve (AUC) of the receiver-operating-characteristic (ROC) curve was computed to evaluate the classification performance of each protein for controls and CIN groups, compared to the CC group. Fold change (FC) was also calculated for these groups. All FC calculations were done using the difference in means of the log2 transformed data and then converting the log2 value to back to its integer value. Cox proportional hazards models were used to evaluate the impact of serum protein levels on OS and PFS. OS was calculated as time (in years) from diagnosis to date of death, and PFS was calculated as time (in years) from diagnosis to the first progression. Patients with no evidence of disease were censored at the date of last follow-up visit. Kaplan-Meier survival analysis and log-rank test were used to compare differences in OS between groups classified based on median protein level.

Paraffin tissue blocks were obtained from each patient's original surgery (CIN and CC) from the Department of Pathology at the Medical College of Georgia. Control cases were obtained from women undergoing total hysterectomy for non-malignant conditions. All immunohistochemistry (IHC) was performed by Georgia Esoteric and Molecular labs (Augusta, GA, USA) and all results were reviewed independently by two pathologists, who were blinded to patient identifiers and clinical results. The primary antibody used was rabbit anti-MMP7.

Results

A total of 63 patients were included in this study. There were 23 invasive squamous cell and adenocarcinoma CC patients, 20 with pre-invasive cervical intraepithelial neoplasia (CIN II/III), and 20 controls. Table 1 summarizes patient demographics. Fourteen of the 23 (61%) patients achieved initial remission and 18/23 (78%) eventually progressed. Not all events were captured for all patients. Eighteen of the patients had diagnosis samples. Ten of the 14 initial remission patients had corresponding blood samples. Twelve of the 18 patients with progression of disease had corresponding blood samples. Two patients were lost to follow up after progression of disease.

Differences in protein expression were compared between controls, CIN, and CC patients divided into three groups: diagnosis, NED, and progression. The distribution of serum protein concentrations was calculated for each protein in each group of patients. Six of the 18 proteins tested had significantly different levels ($p < 0.05$) when patients with CC at diagnosis were compared to both controls and CINs. The six significant proteins and their mean concentration at diagnosis are shown in Table 2. The best five are in bold, with their area under the curve (AUC) value, corresponding *p*-value, and sensitivity at 95% specificity. MMP7 serum concentration was the best indicator of cancer with an AUC value of 0.98. Non-significant ($p > 0.05$) proteins include osteopontin (OPN), vascular endothelial growth factor D (VEGFD), cancer antigen 125 (CA125), cancer antigen 153 (CA153) (soluble vascular cell adhesion molecule-1 (sVCAM1), IGFBP1 IGFBP3, IGFBP6, MMP2, and MMP10.

Figure 1A represents the top five proteins when comparing between controls, CINs, and CC patients at diagnosis.

Table 1. — *Patient demographics.*

		Control (n= 20)	CIN (n=20)	Total CC patients (n=23)	Diagnosis samples (n=18)	Remission samples (n=10)	Progression of disease samples (n=12)
Age	Mean + SD	35 ± 9	38 ± 11	48 ± 14	48 ± 15	47 ± 17	51 ± 16
	Median	34	34	44	44	43	45
	Range	22-47	22-60	28-89	28-89	28-89	35-89
Achieved RM		N/A	N/A	14/23			
Progressed		N/A	N/A	18/23			
Stage		N/A	N/A				
	I	N/A	N/A	7	6	5	2
	II	N/A	N/A	8	6	4	6
	III	N/A	N/A	3	2	0	1
	IV	N/A	N/A	5	4	1	3

Patient demographics. SD = standard deviation, n = number of patients, CIN = pre-invasive cervical intraepithelial neoplasia, CC = invasive cervical cancer.

Table 2. — *Summary of potential cervical cancer serum biomarkers.*

Protein	Protein description	Protein concentration	*p*-value CC at diagnosis *vs.* Ctrl	*p*-value CC at diagnosis *vs.* CINS	AUC (CC at diagnosis *vs.* Ctrl)	*p*-value	Sensitivity at 95% specificity
MMP7 **MMP = matrix metalloprotease**	**Matrix metalloproteases family**	**13.85 pg/ml**	**1.8E-09**	**1.82E-07**	**0.98 (0.96–1)**	**3.71E-07**	**88.90**
IGFBP4 **IGFBP = insulin growth factor binding protein**	**Insulin like growth factor binding protein family**	**15.21 pg/ml**	**0.0005**	**0.003**	**0.81 (0.74–0.88)**	**1.37E-03**	**25.0**
IGFBP7	**Insulin like growth factor binding protein family**	**14.10 pg/ml**	**0.037**	**0.005**	**0.75 (0.67–0.83)**	**8.51E-03**	**38.89**
CEA	**Carcinoembryonic antigen**	**10.16 pg/ml**	**0.025**	**0.026**	**0.74 (0.66–0.82)**	**0.012**	**50**
OPG osteoprotegerin	**Member of tumor necrosis factor receptor family**	**8.57 pg/ml**	**0.0094**	**0.00386**	**0.73 (0.65–0.81)**	**0.017**	**33.33**
CA199	Cancer antigen 199	3.93 U/ml	0.024	0.038	N/A	N/A	N/A

Six proteins that had significant differences when comparing CC (cervical cancer) at diagnosis, to both CIN (cervical intraepithelial lesion), and to ctrls (control). All protein concentrations where in picograms per milliliter (pg/ml) except for CA199 which was reported in units/ml (U/ml). All concentrations were log2 transformed from their original value. The five best proteins are in bold and (MMP7, IGFBP4, IGFBP7, CEA, OPG) were examined for their ability to detect differences between CC at diagnosis and ctrls serum protein concentration, as demonstrated by AUC (area under the curve value), sensitivity, and specificity.

MMP7 had the highest fold change in serum protein level compared to CINs (4.5 times as high) and controls (seven times as high) at diagnosis. Receiver operator characteristic (ROC) was performed between controls, and CC patients at diagnosis for the best five proteins (Figure 1B). Refer to Table 2 for *p*-values related to the AUC, sensitivity, and specificity of the top five proteins as calculated from the ROC. Interestingly, some of these five proteins in Table 2 were also excellent indicators of PFS and OS.

MMP7 serum levels at diagnosis (first serum sample within 30 days of diagnosis) were significant for predicting PFS (HR 3.82, CI: 1.10–13.29 $p = 0.025$). OPG (HR 0.23, CI: 0.07–0.77, $p = 0.01$), and VEGFD (HR 3.43, CI: 1.02–11.54, $p = 0.04$) levels at diagnosis were also indicative of PFS (Figure 2A). However, MMP7 (HR 7.96, CI: 0.91–69.32, $p = 0.03$) and OPN (HR 7.90, CI: 0.89–70.03 $p = 0.03$) levels at diagnosis were the only proteins predictive of OS (Figure 2B). For all PFS and OS proteins, patients were divided into high and low groups based on a 50% cut-off as determined by the median protein concentration for each individual protein.

The differences between protein concentrations were also analyzed between samples at diagnosis, remission, and recurrence. No proteins had significantly different levels at diagnosis and remission. OPN had significantly different levels at progression when compared with remission ($p = 0.041$, fold change (FC) = 2.76). MMP7 trended towards significantly elevated levels at progression when compared to remission ($p = 0.07$, FC = 1.99) (Figure 3). Of note, both OPN and MMP7 had trends, which although not statistically significant, showed that the levels of both proteins decrease in remission compared to diagnosis, but then

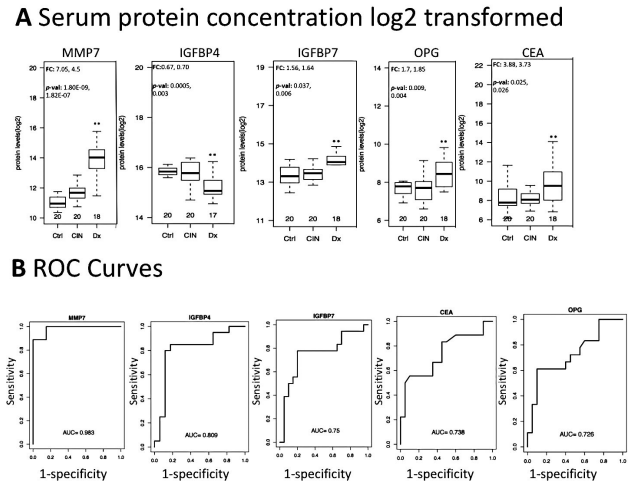

Figure 1. — (A) Protein concentrations of the five best proteins compared between controls, CINs, and cervical cancer patients at diagnosis. Ctrl = control, CIN = pre-invasive cervical intraepithelial neoplasia, Dx = cervical cancer patients at diagnosis. Fold change (FC) and *p*-values (p-val) are also shown on these plots (first value: Dx *vs.* Control; second value: Dx *vs.* CIN. Dx columns with ** above them signify that at Dx the protein level is significantly different compared to both controls and CIN. Median protein concentration is the bold line in each box, the dotted lines extend to the 90th percentile and 10th percentile of serum protein concentration. (B) Receiving-operator-characteristics (ROC) curves that evaluate the ability of these serum proteins to distinguish CC patients at diagnosis from controls. The area under the curve (AUC) for individual proteins were calculated and are shown in the Figure. (ROC= Receiver operator characteristic)

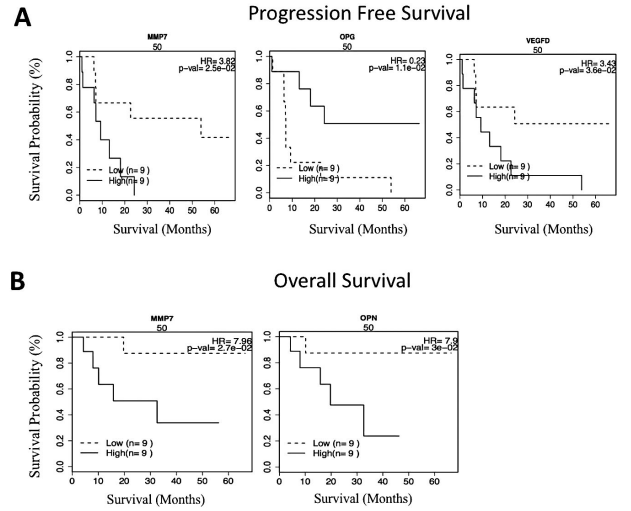

Figure 2. — (A) Differences in progression free survival between the patients with high and low serum concentrations of MMP7, OPG, and VEGFD. The high and low protein serum concentration groups were defined using the median serum protein concentration as cut-off. Hazard ratio (HR) and *p*-values (*p*-val) are shown on top of these plots. (B) Differences in overall survival between high and low serum concentration of MMP7 and OPN. The high and low protein serum concentration groups were defined using the median serum protein concentration as cut-off. Hazard ratio (HR) and *p*-values (*p*-val) are shown on top of these plots.

increased to levels comparable to diagnosis when patients recur.

Because MMP7 showed such a significant fold change in expression between controls, CIN, and CC, immunohistochemistry was done only examining MMP7 protein expression. Invasive CC samples were the only samples to have profound MMP7 staining below the basement membrane, which was consistent with invasion. Importantly, CC cells at metastasis sites also stained positive for MMP7. There was no to minimal staining of MMP7 below the basement membrane in both controls and CIN samples (Figure 4).

Discussion

In this prospective pilot study, MMP7 was identified as a protein that was capable of identifying both invasive disease and predicting disease prognosis. Further supporting the serum data that high MMP7 is associated with invasive CC, IHC demonstrated staining for MMP7 below the basement membrane only in invasive CCs, whereas MMP7 staining was concentrated above the basement membrane in CINs. Metastatic sites of CC also stained positive for MMP7.

MMP7 expression has been previously examined in CC using IHC by many researchers. Although there are conflicting reports in the literature for MMP7 expression in CC, the present data concurs with Herfs *et al.* and Wu *et al.*, who both demonstrated MMP7 expression in CC by IHC [16, 17] Herfs *et al.*, in accord with his IHC data, also showed MMP7 RNA expression in CC [16]. An example of a counter study is Sheu *et al.,* who examined the expression of MMPs by IHC in 31 cases of squamous cell carcinoma of the cervix and 31 cases of CIN lesions [15]. Their group reported that 77% of CC patients did not express MMP7, as well as the majority of CINs showed no MMP7 expression [15]. In the present study all CIN and all CC patients demonstrated MMP7 staining, with only invasive CC patients demonstrating MMP7 staining below the basement membrane. This is very similar to Herfs *et al.* study, which demonstrated MMP7 staining in all cases of CIN 2/3 (48 cases) and all invasive CC patients (19 patients) [16].

Despite the exploration of MMP7 expression in CC by IHC, MMP7 serum expression to the present authors' knowledge has not been explored in CC up until this point; however, high MMP7 serum levels have been shown to be associated with a poor prognoses in both colon and pancreatic cancer [18, 19]. Given the natural role MMPs play in promoting invasion, as well as MMP7's proven association with other cancers, future studies will focus on patient expansion to confirm if MMP7 could serve as a potential marker for invasive CC diagnosis and prognosis. In addition to the strength of MMP7 being associated with invasion and prognosis, the present study also revealed many other proteins which are at play in CC, including

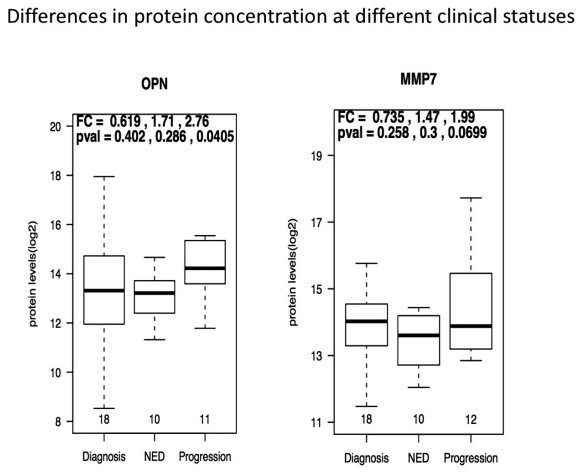

Figure 3. — Differences in protein concentration in patients by clinical status: diagnosis, no evidence of disease, and progression. NED = no evidence of disease. Fold change (FC) and *p*-values (pval) are shown on top of these plots (first value: NED *vs.* diagnosis; second value: progression *vs.* diagnosis; third value: progression *vs.* NED. Median protein concentration is the bold line in each box, the dotted lines extend to the 90th percentile, and 10th percentile of serum protein concentration.

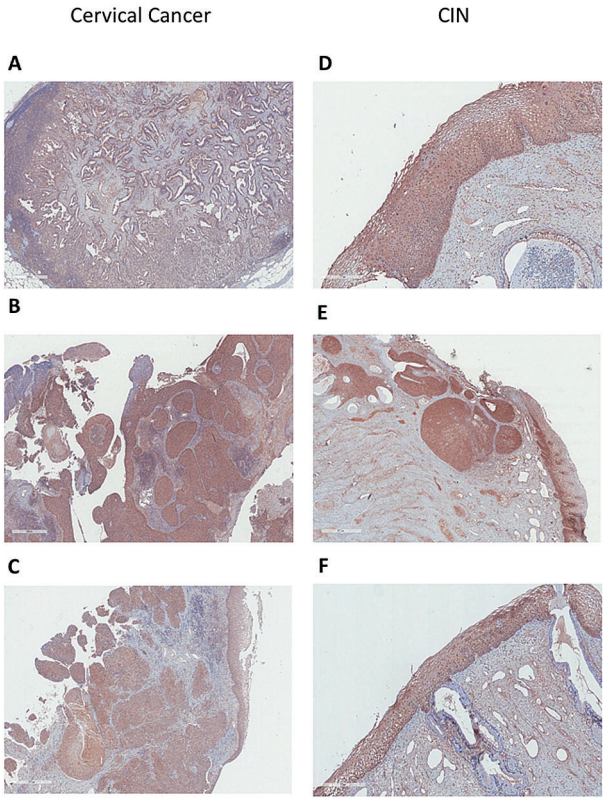

Figure 4. — Immunohistochemistry of both cervical cancer (A-C, ×4 magnification) and CIN (D-F, ×10 magnification) patient samples stained for MMP7 (CIN= cervical intraepithelial neoplasia).

OPG, VEGFD, and OPN.

The present study showed OPG to be significantly lower in patients with decreased OS. OPG, or osteoprotegerin, was first discovered in association with bone growth regulation. High levels of OPG are associated with downregulation of osteoclasts. OPG could possibly serve as an inflammatory mediator in cancer, especially given that it is a member of the tumor necrosis factor receptor family [20]. Part of OPG's role in inflammation includes being a moderator of the RANK/RANKL pathway, which is associated with a poor prognosis in breast cancer. OPG modulates this pathway because is also able to serve as a soluble RANK receptor, thus impairing the RANK/RANKL pathway by binding molecules which would bind to RANK. Both RANK and RANKL have recently been implicated in promoting CC growth, as well as contributing to an immunosuppressive environment [20-23]. This provides support for decreased OPG being associated with poor prognosis, as decreased OPG would mean the RANK/RANKL pathway could be more active.

High levels of VEGFD were also predictive of PFS in the present patient population. This is not surprising, given recent findings that treatment with VEGF receptor blockers, specifically bevacizumab, prolong survival in advanced stage CC patients. Bevacizumab was the first drug approved in 20 years to be used in combination with cisplatin and paclitaxel [3]. Of note, VEGFB and VEGFC have previously been reported to be elevated in patients with CC [14].

OPN has an established role in the immune system, serving as a cell adhesion molecule. This molecule has also been implicated as prognostic biomarker when measured in the plasma of a variety of cancers, including lung and breast cancer [24]. Furthermore, recent studies in mice have shown that monoclonal antibodies targeting OPN are a potential effective treatment [25]. Arguably the most supportive study of OPN's prognostic role in CC was conducted by Huang *et al.*, who demonstrated that increased OPN levels by IHC conferred radiation therapy resistance [26]. This is reassuring when compared with the present data, which demonstrated that increased levels of OPN were indicative of decreased OS and disease progression.

Overall, the present data suggests that serum biomarkers may be a viable option to screen for invasive CC, monitor disease status, and predict prognosis. Furthermore, MMP7 shows specific promise both as a prognostic indicator and a screening a tool. Future studies will focus on expanding patient numbers to verify MMP7's role as a screening and prognostic tool in CC, as well as further investigating other biomarkers' roles as possible prognostic markers.

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References

[1] Ibeanu O.A.: "Molecular pathogenesis of cervical cancer". *Cancer Biol. Ther.*, 2011, *11*, 295.

[2] Franco L.E., Schlecht F.N., Saslow D.: "The epidemiology of cervical cancer". *Cancer J.*, 2003, *9*, 348.

[3] Tewari K.S., Sill M.W., Long H.J. 3rd., Penson R.T., Huang H., Ramondetta L.M., *et al.*: "Improved survival with bevacizumab in advanced cervical cancer". *New Engl. J. Med.*, 2014, *370*, 734.

[4] Partridge E.E., Abu-Rustum N.R., Campos S.M., Fahey P.J., Farmer M., Garcia R.L., *et al.*: "Cervical cancer screening". *J. Natl. Compr. Canc. Netw.*, 2010, *8*, 1358.

[5] Goldie S.J., Kuhn L., Denny L., Pollack A., Wright T.C.: "Policy analysis of cervical cancer screening strategies in low-resource settings: clinical benefits and cost-effectiveness". *JAMA*, 2001, *285*, 3107.

[6] Schiffman M., Castle P.E., Jeronimo J., Rodriguez A.C., Wacholder S.: "Human papillomavirus and cervical cancer". *Lancet*, 2007, *370*, 890.

[7] Giuliano A.R., Palefsky J.M., Goldstone S., Moreira E.D. Jr., Penny M.E., Aranda C., *et al.*: "Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males". *New Engl. J. Med.*, 2011, *364*, 401.

[8] Waggoner S.E.: "Cervical cancer". *Lancet*, 2003, *361*, 2217.

[9] Gadducci A., Cosio S., Carpi A., Nicolini A., Genazzani A.R.: "Serum tumor markers in the management of ovarian, endometrial and cervical cancer". *Biomed. Pharmacother.*, 2004, *58*, 24.

[10] Gadducci A., Tana R., Cosio S., Genazzani A.R.: "The serum assay of tumour markers in the prognostic evaluation, treatment monitoring and follow-up of patients with cervical cancer: a review of the literature". *Crit. Rev. Oncol. Hematol.*, 2008, *66*, 10.

[11] Smith L., Lind M.J., Welham K.J., Cawkwell L., Cancer Biology Proteomics Group: "Cancer proteomics and its application to discovery of therapy response markers in human cancer. *Cancer*, 2006, *107*, 232.

[12] Eisenhauer E.A., Therasse P., Bogaerts J., Schwartz L.H., Sargent D., Ford R., *et al.*: "New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1)". Eur. J. Cancer, 2009, *45*, 228.

[13] Huang Y.F., Shen M.R., Hsu K.F., Cheng Y.M., Chou C.Y.: "Clinical implications of insulin-like growth factor 1 system in early-stage cervical cancer". *Br. J. Cancer*, 2008, *99*, 1096.

[14] Mathur S.P., Mathur R.S., Gray E.A., Lane D., Underwood P.G., Kohler M., *et al.*: "Serum vascular endothelial growth factor C (VEGF-C) as a specific biomarker for advanced cervical cancer: Relationship to insulin-like growth factor II (IGF-II), IGF binding protein 3 (IGF-BP3) and VEGF-A [corrected]". *Gynecol. Oncol.*, 2005, *98*, 467.

[15] Sheu B.C., Lien H.C., Ho H.N., Lin H.H., Chow S.N., Huang S.C., *et al.*: "Increased expression and activation of gelatinolytic matrix metalloproteinases is associated with the progression and recurrence of human cervical cancer". *Cancer Res.*, 2003, *63*, 6537.

[16] Herfs M., Yamamoto Y., Laury A., Wang X., Nucci M.R., McLaughlin-Drubin M.E., *et al.*: "A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer". *Proc. Natl. Acad. Sci. U S A*, 2012, *109*, 10516.

[17] Wu S.H., Zhang J., Li Y., Li J.M.: "Expression of ETV5 and MMP-7 in early stage cervical squamous cell carcinoma and its role in lymphatic metastasis". *Ai Zheng*, 2006, *25*, 315.

[18] Klupp F., Neumann L., Kahlert C., Diers J., Halama N., Franz C., *et al.*: "Serum MMP7, MMP10 and MMP12 level as negative prognostic markers in colon cancer patients". *BMC Cancer*, 2016, *16*, 494.

[19] Wang S.C., Parekh J.R., Porembka M.R., Nathan H., D'Angelica M.I., DeMatteo R.P., *et al.*: "A Pilot Study Evaluating Serum MMP7 as a Preoperative Prognostic Marker for Pancreatic Ductal Adenocarcinoma Patients". *J. Gastrointest. Surg.*, 2016, *20*, 899.

[20] Renema N., Navet B., Heymann M.F., Lezot F., Heymann D.: "RANK-RANKL signalling in cancer". Biosci. Rep., 2016, 36, pii: e00366.

[21] Demoulin S.A., Somja J., Duray A., Guenin S., Roncarati P., Delvenne P.O., *et al.*: "Cervical (pre)neoplastic microenvironment promotes the emergence of tolerogenic dendritic cells via RANKL secretion". *Oncoimmunology*, 2015, *4*, e1008334.

[22] Shang W.Q., Li H., Liu L.B., Chang K.K., Yu J.J., Xie F., *et al.*: "RANKL/RANK interaction promotes the growth of cervical cancer cells by strengthening the dialogue between cervical cancer cells and regulation of IL-8 secretion". *Oncol. Rep.*, 2015, *34*, 3007.

[23] Yoldi G., Pellegrini P., Trinidad E.M., Cordero A., Gomez-Miragaya J., Serra-Musach J., *et al.*: "RANK signaling blockade reduces breast cancer recurrence by inducing tumor cell differentiation". *Cancer Res.*, 2016, *76*, 5857.

[24] Weber G.F., Lett G.S., Haubein N.C.: "Osteopontin is a marker for cancer aggressiveness and patient survival". *Br. J. Cancer*, 2010, *103*, 861.

[25] Maeda N., Ohashi T., Chagan-Yasutan H., Hattori T., Takahashi Y., Harigae H., *et al.*: "Osteopontin-integrin interaction as a novel molecular target for antibody-mediated immunotherapy in adult T-cell leukemia". *Retrovirology*, 2015, *12*, 99.

[26] Huang X., Qian Y., Wu H., Xie X., Zhou Q., Wang Y., *et al.*: "Aberrant expression of osteopontin and E-cadherin indicates radiation resistance and poor prognosis for patients with cervical carcinoma". *J. Histochem. Cytochem.*, 2015, *63*, 88.

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