Heliyon

ELSEVIER

Received:
4 May 2016
Revised:
5 July 2016
Accepted:
10 August 2016

Heliyon 2 (2016) e00143

Intra-tumour IgA1 is common in cancer and is correlated with poor prognosis in bladder cancer.

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Abstract

A high frequency of IgA1-positive tumour cells was found in tissue micro-arrays of oesophagus, colon, testis, lung, breast, bladder and ovarian cancer. IgA1 was observed in the cytoplasm and the plasma membrane. A correlation was found between intra-tumour IgA1 and poor overall survival in a large cohort of bladder cancer patients ($n = 99$, $p = 0.011$, log-rank test). The number of IgA1-positive tumour cells was also found to be higher in female than male bladder cancer patients. The presence of IgA1 was confirmed in formalin-fixed paraffin-embedded ovarian carcinoma samples using LC-MS/MS analysis. Uptake of IgA1 was also observed in breast cancer and melanoma cell lines when cultivated in the presence of serum from healthy individuals, indicating a possible origin of the IgA1 antibodies in cancer cells.

Keyword: Medicine

http://dx.doi.org/10.1016/j.heliyon.2016.e00143

1. Introduction

There are reports in the literature of increased concentrations of the secretory component of immunoglobulin A (IgA) and a higher expression of IgA and IgM in serum from patients with cancer [1, 2]. The presence of IgA1 in preparations from neoplasms of epithelial origin was first reported in 1996 by Streets et al. [3]. When analysing Helix pomatia agglutinin-binding glycoproteins from primary breast cancers, they found IgA1 to be a major component among the Tn antigen- (O-linked serine/threonine-alpha GalNac) positive proteins. In a previous study we confirmed the high frequency of IgA1 staining in breast cancer tissue sections, where we found clearly IgA1-positive but heterogeneous intra-tumour staining [4]. Invasive parts of the tumours were found to be more intensively stained than the *in situ* component, and both the cytoplasm and the plasma membrane were stained. In this study we wanted to investigate if the expression of IgA1 was exclusive for breast carcinoma or if IgA1 also were expressed in other forms of carcinomas. The origin of tumour-associated immunoglobulins is not known, but there are several kinds of receptors for IgA uptake [5], suggesting receptor-mediated uptake from surrounding blood plasma as an alternative to when synthesized and released antigens binds to IgA into a complex and then by endocytosis enters the cell [6]. In this study, we investigated the frequency of tumour-associated IgA1 in a number of different cancers, and investigated the relationship between IgA1 and clinical outcome in a large cohort of bladder cancer patients.

2. Subjects and methods

2.1. Subjects

Tumours are from oesophagus (n = 12), colon (n = 48), testis (n = 57), lung (n = 12) breast (n = 52), ovarian (n = 50) and bladder (n = 110). The present study was approved by the Ethics Committee at Lund University (Ref. 445/2007) and informed consent was obtained from all patients.

2.2. Tissue micro-array construction

All the tumours were histopathologically re-evaluated and classified according to the WHO grading system of 2004 by a board-certified pathologist before tissue micro-array (TMA) construction. Areas representative of the cancer were then marked, and TMAs were constructed as described previously [7]. Briefly, two tissue cores were taken from each tumour and mounted in a new recipient block using a semi-automated arraying device (TMArrayer, Pathology Devices, Inc., Westminster, MD, USA).

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2.3. Immunohistochemistry

For immunohistochemistry (IHC) analysis, 4-μm TMA sections were automatically pretreated using the PT Link system (DAKO, Glostrup, Copenhagen, Denmark), and then stained in an Autostainer Plus (DAKO, Glostrup, Copenhagen, Denmark) with the primary antibody M4D8 anti-human IgA1 (dilution 1:3000) obtained from Margaret Goodall at The School of Immunity & Infection, Birmingham University (UK). The specificity of the antibody has been demonstrated previously [8].

2.4. Bladder cancer patients

Consecutive patients diagnosed with urothelial bladder cancer at the Department of Pathology, Skåne University Hospital, Malmö, from 1 October 2002 until 31 December 2003, for whom archival transurethral resection specimens of the bladder could be retrieved were included in the cohort (n = 110). The cohort included 80 men (72.7%) and 30 women (27.3%), and the median age was 72.9 years (range 39.3–89.9 years). Information on vital status was obtained from the Swedish Cause of Death Register up to 31 December 2010. Follow-up started at the date of diagnosis and ended at death, emigration or on 31 December 2010, whichever was first. The median follow-up time was 5.92 years (range 0.03–8.21 y) for the whole cohort, and 7.71 years (range 7.04–8.21 y) for patients alive (n = 48) on 31 December 2010. Forty-eight patients (43.6%) died within 5 years. The T-stage distribution of the tumours was: 48 (43.6%) pTa, 24 (21.8%) pT1, 37 (33.8) pT2 and 1 (0.9%) pT3. Eighteen (16.4%) tumours were Grade I, 34 (30.9%) Grade II and 58 (52.7%) Grade III. This cohort has also been described previously [9, 10, 11]. Following antibody optimisation and staining, IgA1 expression could be evaluated in 99 out of 110 tumours (90%). Those that could not be evaluated were either the result of complete tissue loss during IHC preparation or an insufficient quantity of tumour tissue during IHC preparation. The expression of IgA1 was assessed as the staining of the cytoplasma and then categorized into five groups: 0 (0%–1%), 1 (2%–25%), 2 (26%–50%), 3 (51%–75) and 4 (>75%). The cytoplasmic staining intensity was also noted as 0 = negative, 1 = intermediate, 2 = moderate and 3 = strong intensity. A combined score was then obtained by multiplying the staining scores of IgA1 by the staining intensity, resulting in categories ranging from 0 to 12. For survival analysis, this variable was dichotomized into low (categories 0–2) and high (categories 3–12) IgA1-expressing groups.

2.5. Statistical analysis for bladder cancer

To test for correlations between IgA1 and clinical parameters, Spearman's rank correlation coefficient (rho, ρ) was used. Overall survival was investigated using Kaplan-Meier analysis combined with the log-rank test to elucidate differences in

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outcome. Cox regression analysis was performed to estimate the relationship between survival and IgA1 status, unadjusted and adjusted for age, grade and T-stage. All statistical analyses were carried out using IBM SPSS Statistics version 23 (IBM Corporation, Armonk, NY, USA). All p-values were two-sided and differences were considered significant when $p < 0.05$.

2.6. Tissue excision from ovarian cancer tumours and protein digestion

Nine cores were excised from the formalin-fixed paraffin-embedded blocks from nine patients with ovarian cancer, and transferred to Eppendorf tubes. The nine samples were taken from sections with low (T4080, T22530 and T13975), medium (T3260, T10387 and T12696) or high (T195, T15392 and T2852) expression of IgA1 based on IHC. The cores were deparaffinised using 1 mL EnVision™ FLEX target retrieval solution (High pH) (Dako, Glostrup, Denmark) and heated for 10 min at 98 °C. Samples were centrifuged for 10 min at 14000 x g at 4 °C. The paraffin, floating on the surface, was removed, and the retrieval solution was aspirated. Another mL of fresh retrieval solution was added to the samples, which were heated for another 10 min at 98 C followed by centrifugation for 10 min at 14000 x g at 4 °C. The retrieval solution was again aspirated. To these tissue samples, 300 μL 6 M guanidine chloride in 50 mM ammonium bicarbonate was then added. The samples were sonicated for 5 min with a Branson SLPe sonicator (Branson Ultrasonics, Danbury, CT, USA) in continuous mode. The samples were then centrifuged at 14000 x g for 10 min at room temperature. The supernatants were transferred to new tubes and the pellets were discarded. The protein concentration was measured using a Bicinchoninic acid protein assay according to the manufacturer's instructions (Micro BCA kit, Pierce, Thermo Scientific, Rockford, IL, USA). The samples, 150 μg proteins, were reduced with dithiothreitol and alkylated with iodoacetic acid before trypsination, as described previously [12].

2.7. Label free quantification by LC-MS/MS analysis

Samples, 1 μg were analysed using LC-MS/MS in an Easy-nLC II system coupled to a QExactive mass spectrometer (Thermo Scientific, San José, CA, USA). The peptides were concentrated (on-line) by reverse-phase chromatography using a 20 mm x 0.75 mm, 3 μm C18 RP pre-column (Acclaim PepMap® 100, nanoViper), and then separated using a 250 mm x 0.075 mm, C18, 2 μm, 100 Å column (Acclaim PepMap® 100 RSLC, nanoViper) at a flow rate of 300 nL/min, both from Thermo Scientific (San José, CA, USA).

Raw data files were analysed with Proteome Discoverer v 1.4 (Thermo Scientific). Peptides were identified using SEQUEST HT against the UniProtKB human

http://dx.doi.org/10.1016/j.heliyon.2016.e00143

database integrated into Proteome Discoverer. Two unique peptides, DASGVTFTWTPSSGK and TFTCTAAYPESK, were selected for IgA1. MS1 full scan filtering chromatogram-based quantification was performed in Skyline version 3.1 (http://proteome.gs.washington.edu/software/skyline) [13]. Quantitative analysis was based on extracted ion chromatograms (XICs) and resulting precursor ion peak areas for each peptides M, M+1 and M+2, *i.e*. the first, second and third isotopic envelope. The sample digests were then subjected to new targeted scheduled LC-MS/MS runs with an isolation list of the precursors and with a time window of 2 min. Data from the individual tumour lysates are presented as mean of triplicates measurements +/− standard deviation.

2.8. Culturing and analysis of melanoma and breast cancer cells

The cell lines, T47D (breast cancer) and C8161 (melanoma) were cultured close to confluency at 37 °C in an atmosphere of 5% CO_2 in RPMI medium supplemented with GlutaMAX™, 10% foetal calf serum (FCS), penicillin (100 μg/mL) and streptomycin (100 μg/mL). In half of the cultures the medium was changed to RPMI 1640 medium supplemented with 75% pooled human serum from healthy blood donors. These cells were cultured for 24 h. Cells were harvested by 5 min' incubation with trypsin/EDTA at 37 °C. The cells were then washed three times with PBS before fixation with 2% paraformaldehyde and permeabilized with 0.1% saponin, 5% BSA in PBS. The cells were either stained with the primary antibody anti-IgA1 (M4D8) or a negative control mouse IgG antibody (2 μg/mL) for 1 h, followed by three washes with PBS. As secondary antibody, a FITC-labelled anti-mouse Ig (F0261, Dako) diluted 1/500 was used. The cells were analysed for the presence of IgA1 uptake using an ImageStream® Mark-II imaging flow cytometer (Amnis, Seattle, WA, USA).

3. Results

3.1. Presence of IgA1 in different cancer tissues

Most of the tumour samples investigated, from all tumour types i.e., oesophagus, ovarian, testis, bladder, lung, breast and colon cancer, stained positive for IgA1 (Table 1). IgA1 was frequently seen in both the cytoplasm and plasma membrane of the cancer cells, as can be seen in Fig. 1.

3.2. Findings related to bladder cancer

A larger number of cases of bladder cancer (n = 99) were evaluated to investigate the possible correlation between IgA1 score and patient outcome. A high IgA1score (i.e. many and/or intensively stained IgA1-positive cells) was significantly correlated with gender in that tumours from female patients were more frequently IgA1-positive than tumours from male patients (Table 2). High

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Table 1. A summary of IgA1-positive and -negative cancer TMA samples in seven common kinds of cancer.

Tumour	n	Positive	Negative
Oesophagus	12	10	2
Ovarian	50	47	3
Testis	57	52	5
Bladder	99	61	38
Lung	12	12	0
Breast	52	46	6
Colon	48	38	10

tumour-specific IgA1 expression was also correlated with significantly poorer overall survival (Fig. 2). When adjusting for patient age, and tumour grade and T-stage in a multivariable Cox analysis, age remained the strongest parameter

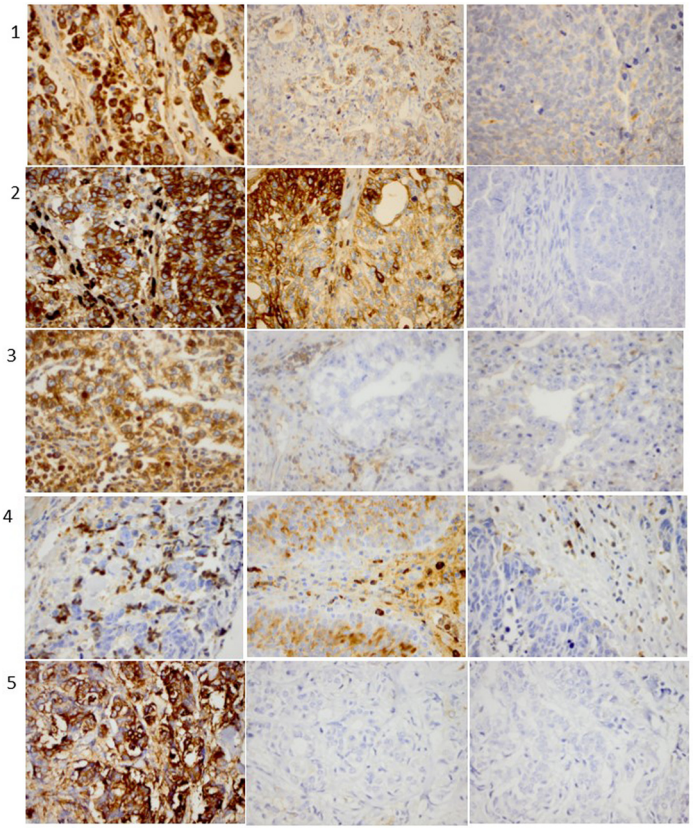

Fig. 1. Images of tumour sections after immunohistochemical staining for IgA1 from1) oesophagus, 2) ovarian, 3) testis, 4) bladder and 5) breast cancer tissue. Tissue sections are from individual patients illustrating the differences in staining intensity.

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Table 2. Expression of IgA1 expression in bladder cancer.

		IgA1[a]	
Factor	**N**	**rho**	**P value**
Sex[b]	99	-0.296	0.003
Grade[c]	99	0.178	0.079
Stage[d]	99	0.262	0.009

N = number of patients.

[a] Staining x intensity.

[b] Female vs. Male.

[c] I, II and III.

[d] pTa, pT1, pT2 and pT3.

predicting overall survival, and the significance of IgA1 expression was lost (Table 3).

3.3. Ovarian tumours

The relative amount of IgA1 in the nine samples of ovarian tumours was quantified using two unique peptides, DASGVTFTWTPSSGK and TFTCTAAYPESK, from the protein. Data from the individual tumour lysates are presented as the mean of triplicate measurements ± standard deviation (Fig. 3). The IgA1 staining of the same tumour is shown in Fig. 4.

3.4. Breast cancer and melanoma cells

Breast cancer and melanoma cells cultivated in FCS stained negative for IgA1 using flow cytometry. However, cells cultivated in human serum stained positive

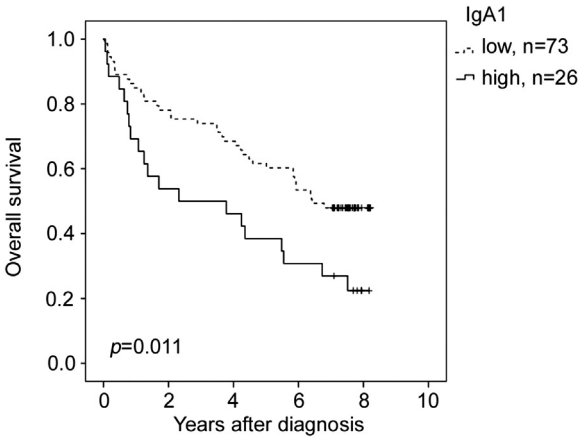

Fig. 2. Kaplan-Meier plot of the overall survival in 99 patients with bladder cancer. A high IgA1 score is associated with poorer overall survival (p = 0.011, log-rank test).

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Table 3. Uni- and multivariable Cox regression analysis of overall survival in the bladder cancer cohort (N = 99).

	Univariable			Multivariable		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Age[a]	1.08	1.05 to 1.10	<0.001	1.07	1.04 to 1.10	<0.001
Grade[b]	3.70	2.13 to 6.44	<0.001	1.44	0.63 to 3.30	0.387
T-stage						
pTa	1.00			1.00		
pT1	4.12	2.09 to 8.11	<0.001	2.60	1.09 to 6.17	0.032
pT2 + 3	4.13	2.19 to 7.80	<0.001	2.24	0.85 to 5.90	0.104
IgA1[c]	1.99	1.16 to 3.43	0.013	1.79	0.99 to 3.24	0.056

HR = hazard ratio.
CI = confidence interval.
[a] Continuous.
[b] Grade I-II vs. III.
[c] IgA1 low vs. high.

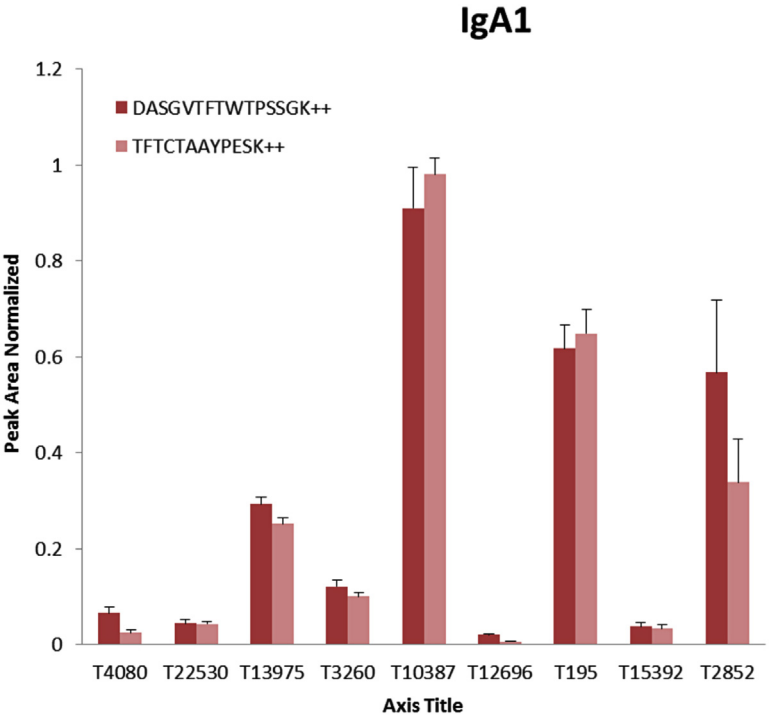

Fig. 3. IgA1 expression levels of the two selected peptides in nine individual patient ovarian cancer tissue samples using LC-MS/MS. The results are given as the mean and SD of triplicate measurements.

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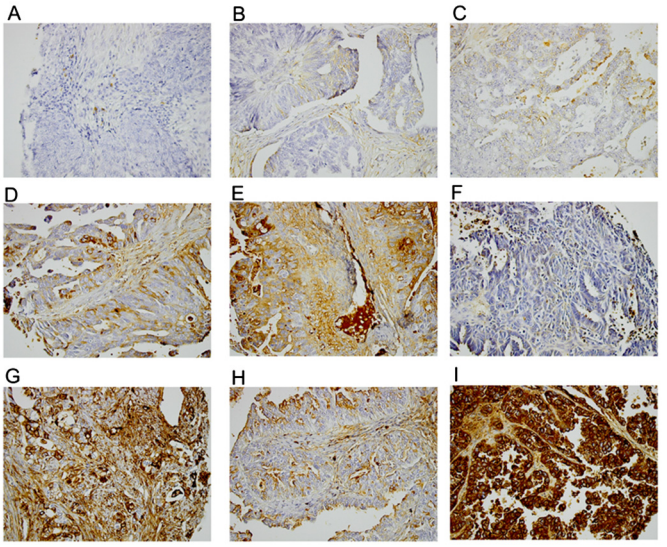

Fig. 4. Images of ovarian tumour sections after immunohistochemical staining for IgA1. Section with low A) T4080, B) T22530, C) T13975, medium D) T3260 E) T10387, F) T12696 and high G) T195, H) T15392 and I) T2852 expression of IgA1.

for IgA1 when the cells had been fixated and permeabilized, clearly showing an uptake of IgA1 (Fig. 5).

4. Discussion

The aim of the present study was to investigate the frequency of IgA1-positive cells in tumours from different types of cancer. It is clear from the results that the presence of IgA1 in cancer cells is a common phenomenon. In the seven types of tumours investigated, over 80% of the tumour samples stained positive for IgA1. The score for IgA1-positive cancer cells in patients with bladder cancer ($n = 99$) was correlated with the tumour stage and overall survival, indicating a relation between the presence of IgA1 in cancer cells and poor prognosis. Patients with high IgA1-expressing tumours had a significantly lower overall survival than those with low IgA1-expressing tumours. When adjusting for known prognostic factors such as age, this association was lost.

The presence of IgA was confirmed in tissue sections from ovarian cancer; however, the relative quantities determined with LC-MS/MS were not always correlated with the corresponding IHC (Fig. 3 and Fig. 4). For example tumour T2852 obtained the most intense IgA1 staining with IHC, but not the highest relative quantity when analysed using LC-MS/MS. This could be explained by the heterogeneity of staining seen in whole histological sections [14].

Neither the origin nor function of the immunoglobulins found in cancer cells is known. Apart from possible immune responses to the tumour, uptake by a number

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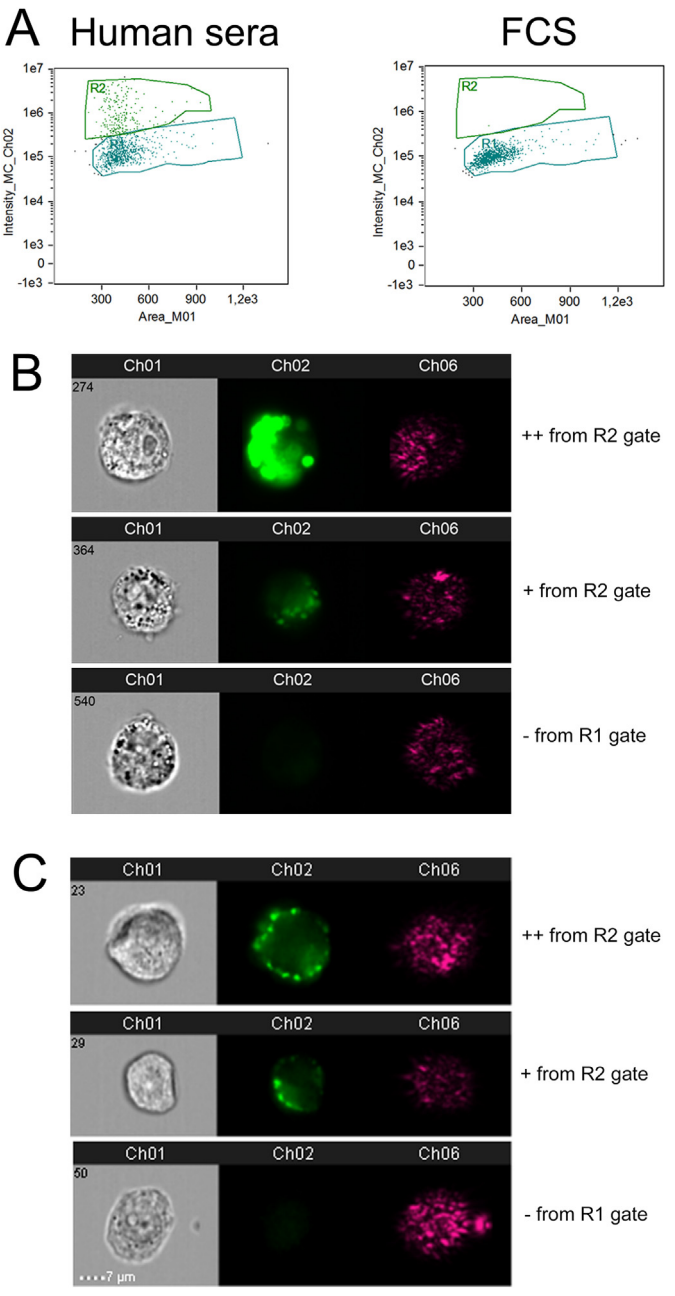

Fig. 5. Image stream analysis of the presence of IgA1 in human cell lines T47D (breast cancer) and C8161 (melanoma) cultured in human serum and in FCS. Fixed cells were permeabilized and stained with anti IgA1 antibody and visualized with a secondary FITC-labelled anti-mouse Ig antibody. A) The intensity of the IgA1 fluorescence signal plotted against the relative cell area of C8161 cells cultivated in human serum or foetal calf serum. R2 indicates the gate with cells regarded as positively stained for IgA1 uptake. Three representative individual B) C8161 cells C), and T47D cells after culture in human serum showing strong (+++), weak (+) and no staining (−) for IgA1. Channel 1 (Ch01) bright field, channel 2 (Ch02) FITC and IgA1 uptake, channel 6 (Ch06) represent side scatter.

http://dx.doi.org/10.1016/j.heliyon.2016.e00143

of receptors may be possible; Fc alphaRI (CD89), pIgR, Fc α/μR, asialoglycoprotein receptor and the transferrin receptor all with the capacity to transfer IgA1 into the cell [5]. A specific immune response to tumours could result in the uptake of immunoglobulins in a similar way to that observed in virus-infected cells [6]. IgA, IgM and IgG can all enter into virus infected cells *in vitro* and via the high affinity IgG receptor, TRIM21, the immunoglobulins can then bind and support destruction of virus inside the cell [15]. It might be possible that anti-tumour antibodies can act in a similar way through TRIM21 by destroying the antigen and perhaps promoting tumour growth. When cultivating cell lines which were found to be IgA1-negative in the presence of human serum, a portion of the cells showed an uptake of IgA1 after 24 h, which demonstrates the capability of tumour cells to internalize IgA1 from serum.

It has been suggested that the presence of immunoglobulins in tumour cells is due to the fact that non-lymphoid cells, including cancer cells, express immunoglobulins [16, 17, 18]. However, we were not able to confirm any production of immunoglobulins by tumour cell lines in the present study or in our previous study [4]. Neither could Babbage et al. [19] detect any IgA chain on protein level after sorting EpCAM-positive epithelial cancer cells using FACS, although they did detect Ig on the mRNA level.

In conclusion, IgA1 is present in tumour cells in a wide range of cancers, and the presence of IgA1 in tumour cells is correlated with poor prognosis in bladder cancer. However, the origin and function of immunoglobulins must be further investigated.

Declarations

Author contribution statement

Charlotte Welinder, Bo Jansson: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Karin Jirström: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Sophie Lehn: Analyzed and interpreted the data.

Björn Nodin: Performed the experiments; Analyzed and interpreted the data.

György Marko-Varga, Ola Blixt, Lena Danielsson: Contributed reagents, materials, analysis tools or data.

Funding statement

The authors received funding from Mrs Berta Kamprad's Foundation.

http://dx.doi.org/10.1016/j.heliyon.2016.e00143

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] J.P. Harris, M.H. Caleb, M.A. South, Secretory component in human mammary carcinoma, Cancer Res. 35 (7) (1975) 1861–1864.

[2] M.M. Roberts, E.M. Bathgate, A. Stevenson, Serum immunoglobulin levels in patients with breast cancer, Cancer 36 (1) (1975) 221–224.

[3] A.J. Streets, S.A. Brooks, M.V. Dwek, A.J. Leathem, Identification, purification and analysis of a 55 kDa lectin binding glycoprotein present in breast cancer tissue, Clin. Chim. Acta 254 (1) (1996) 47–61.

[4] C. Welinder, B. Baldetorp, O. Blixt, D. Grabau, B. Jansson, Primary breast cancer tumours contain high amounts of IgA1 immunoglobulin: an immunohistochemical analysis of a possible carrier of the tumour-associated Tn antigen, PloS one 8 (4) (2013) e61749.

[5] I.C. Moura, M. Arcos-Fajardo, C. Sadaka, V. Leroy, M. Benhamou, J. Novak, et al., Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy, J. Am. Soc. Nephrol. 15 (3) (2004) 622–634.

[6] M.B. Mazanec, C.S. Kaetzel, M.E. Lamm, D. Fletcher, J.G. Nedrud, Intracellular neutralization of virus by immunoglobulin A antibodies, Proc. Natl. Acad. Sci. U S A 89 (15) (1992) 6901–6905.

[7] J. Kononen, L. Bubendorf, A. Kallioniemi, M. Barlund, P. Schraml, S. Leighton, et al., Tissue microarrays for high-throughput molecular profiling of tumor specimens, Nat. Med. 4 (7) (1998) 844–847.

[8] M.A. Farris, D. Hardie, G. de Lange, R. Jefferis, Immunogenic and antigenic epitopes of immunoglobulins X: Monoclonal antibodies specific for human IgA, the IgA1 and IgA2 subclasses and an nA2m(2) iso-allotypic epitope, Vox Sang. 48 (2) (1985) 116–121.

[9] G. Andersson, C. Wennersten, A. Gaber, K. Boman, B. Nodin, M. Uhlen, et al., Reduced expression of ezrin in urothelial bladder cancer signifies more advanced tumours and an impaired survival: validatory study of two independent patient cohorts, BMC Urol. 14 (2014) 36.

http://dx.doi.org/10.1016/j.heliyon.2016.e00143

[10] C. Wennersten, G. Andersson, K. Boman, B. Nodin, A. Gaber, K. Jirstrom, Incident urothelial cancer in the Malmo Diet and Cancer Study: cohort characteristics and further validation of ezrin as a prognostic biomarker, Diagn. Pathol. 9 (2014) 189.

[11] K. Boman, A.H. Larsson, U. Segersten, E. Kuteeva, H. Johannesson, B. Nodin, et al., Membranous expression of podocalyxin-like protein is an independent factor of poor prognosis in urothelial bladder cancer, Br. J. cancer 108 (11) (2013) 2321–2328.

[12] C. Welinder, G.B. Jonsson, C. Ingvar, L. Lundgren, B. Baldetorp, H. Olsson, et al., Analysis of Alpha-Synuclein in Malignant Melanoma – Development of a SRM Quantification Assay, PloS one 9 (10) (2014) e110804.

[13] A. Prakash, D.M. Tomazela, B. Frewen, B. Maclean, G. Merrihew, S. Peterman, et al., Expediting the development of targeted SRM assays: using data from shotgun proteomics to automate method development, J. Proteome Res. 8 (6) (2009) 2733–2739.

[14] K.D. Awadelkarim, C. Arizzi, E.O.M. Elamin, I. Osman, S.O. Mekki, I. Biunno, et al., Tissue Microarray (TMA) Versus Whole Section Immunohistochemistry in the Assessment of ER/PR and Her-2/neu Status in a Breast Cancer Series from Sudan, Breast J. 19 (4) (2013) 446–447.

[15] S. Foss, R. Watkinson, I. Sandlie, L.C. James, J.T. Andersen, TRIM21: a cytosolic Fc receptor with broad antibody isotype specificity, Immunol. Rev. 268 (1) (2015) 328–339.

[16] Z. Chen, X. Qiu, J. Gu, Immunoglobulin expression in non-lymphoid lineage and neoplastic cells, Am. J. Pathol. 174 (4) (2009) 1139–1148.

[17] L.Y. Geng, Z.Z. Shi, Q. Dong, X.H. Cai, Y.M. Zhang, W. Cao, et al., Expression of SNC73, a transcript of the immunoglobulin alpha-1 gene, in human epithelial carcinomas, World J. Gastroenterol. 13 (16) (2007) 2305–2311.

[18] J.B. Hu, S. Zheng, Y.C. Deng, Expression of a novel immunoglobulin gene SNC73 in human cancer and non-cancerous tissues, World J. Gastroenterol. 9 (5) (2003) 1054–1057.

[19] G. Babbage, C.H. Ottensmeier, J. Blaydes, F.K. Stevenson, S.S. Sahota, Immunoglobulin heavy chain locus events and expression of activation-induced cytidine deaminase in epithelial breast cancer cell lines, Cancer Res. 66 (8) (2006) 3996–4000.

 http://dx.doi.org/10.1016/j.heliyon.2016.e00143