ORIGINAL RESEARCH ARTICLE

GLUT 1 receptor expression and circulating levels of fasting glucose in high grade serous ovarian cancer[†]

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Abstract

In recent years, the poorly remarkable goals achieved in terms of patients' important outcomes for ovarian cancer have fueled our interest towards the study of its metabolic roots. Within this research pipeline, we assessed the association between the expression of the glucose transporter GLUT1, as expressed at the tumor tissue level, and circulating pre-surgical levels of fasting glucose in a case series including data from 40 patients with high FIGO stage serous ovarian cancer. Patients who provided data to the current analysis were randomly selected from a larger cohort. To our purposes, the procedures related to serum and tissue collection, storage and biomarker assessment were highly standardized and centralized at the institutional laboratories. The GLUT1 antibody SPM498 SPRING (REF. E13810) was used at a 1:500 dilution in 2 μm slides. Staining for GLUT1 was observed at the cell membrane level in all the cases assessed, but strong staining was described in 29 (72.5) of them. The agreement between the two independent reviewers was 100%. Strong GLUT1 staining was inversely associated with the circulating levels of fasting glucose, with a particularly striking difference in the distribution for patients in the lowest fasting glucose tertile (p=0.044). These results support the biological plausibility of the association of interest. If confirmed in larger studies, our findings may help clarify the potentials of biomarkers related to energy metabolism in terms of prognosis definition, treatment assignment and outcome interpretation for patients with high FIGO stage serous ovarian cancer. This article is protected by copyright. All rights reserved

Introduction

In Western countries, ovarian cancer (OvCa) is sadly renown because of its highly lethal potentials, which have been barely contained over the past decades (Ferlay et al. 2015). The vast majority of OvCa patients are diagnosed with serous, high grade, high stage epithelial carcinoma. As such, these patients are managed throughout a combined approach including surgery and platinum-based regimens. Recent achievements of significant relevance include, though are not limited to, the addition of bevacizumab to the paclitaxel plus carboplatin regimen concurrently and then additionally as single-agent consolidation and maintenance therapy (Burger et al. 2011, Stuart et al. 2011), along with the approval of olaparib in BRCA defective OvCa patients (Kim et al. 2015, Liu & Matulonis 2016). However, a dramatic amelioration of patient important outcomes is tightly linked to the understanding of determinants and mechanisms underlying OvCa onset and progression.

In a recently published work from our research group with a focus on the potential role of metabolic and anthropometrics determinants on ovarian carcinogenesis, we observed an inverse association between fasting glucose and FIGO stage at diagnosis in 147 non diabetic OvCa patients treated with platinum based regimens and/or surgery at our Institute. This association was confirmed in subgroup analysis including body mass index (BMI) data (Vici et al. 2016). On an completely speculative ground, we hypothesized that the fluctuation of circulating glucose levels may be related to differences in the expression of the transporter protein 1 (GLUT1) in OvCa patients with advanced FIGO (International Federation of Gynecolists and Obstetrics) stage at diagnosis. Lower levels of fasting glucose and higher expression rates of GLUT 1 in ovarian cancer tissues may be indicative at the systemic and local level of a sort of adaption of the host organism to the enhanced energy necessities dictated by the ongoing carcinogenetic process.

To test our hypothesis, we have now assessed GLUT1 expression at the OvCa tissue levels in a sub-cohort of randomly selected patients from a group of patients with clinically annotated tissue samples and for whom centrally measured fasting glucose levels were available.

Patients and Methods

We analyzed data related to a subgroup of 40 OvCa patients diagnosed and treated at the Regina Elena National Cancer Institute. The list of patients whose tissues were immonostained was randomly selected from an initial pool of 147 women who represented the overall cohort whose data were analyzed in our prior study.

Archival, routinely processed, formalin fixed, paraffin-embedded (FFPP) surgical pathology specimens from 40 patients diagnosed with serous cystadenocarcinomas were assessed. GLUT1 transporter was detected by immunohistochemistry using the labeled streptavidinbiotin procedure.10–12 Sections were deparaffinized in xylene, and cells were rehydrated in decreasing ethanol solution. Endogenous peroxidase was neutralized using 6% hydrogen peroxide for 3 min. The 2 μm slides were exposed to heat-induced antigen retrieval using a steamer, to be further washed and incubated for 30 min at 22°C with a GLUT1. Tissues were counterstained with hematoxylin. All slides were examined by light microscopy. Two pathologists independently reviewed the slides and the involvement of a third reviewer was foreseen in case of disagreement. The GLUT1 antibody (Ab) SPM498 SPRING (REF. E13810) was used at a 1:500 diluition. To the purposes of our research, the control case was represented by tissue specimen from a cystoadenoma case. All sections were analyzed. Intensity of staining was quantified using a 0 to 3 score with regards to the percentage of position marking at high magnification: no marking (0); less than 20% (1+); 20–50% (2+); and more than 50% (3+).

Descriptive statistics were performed for all the variables of interest, including demographics, reproductive history, life-style habits, anthropometrics, disease grade and stage and modalities of surgical managements. Means and standard deviations (SD) were used to report on continuous variables, while categorical data were rendered by crude numbers and percentages. Categories of immunostaining were compared across strata of fasting glucose levels using the Chi square test. The cut off values for tertile definition were computed at the study population level. Statistical analyses were performed by SPSS software, version 21.

Results

The present analysis includes data from 40 OvCa patients diagnosed and treated at the Regina Elena Cancer Institute. This study population represents a randomly selected subgroup of patients from a wider cohort including 147 OvCa patients. The characteristics of the larger cohort were diffusely reported elsewhere (6). We now report on the subsets of patients, whose main features are summarized in table 1.

In our case series, mean age at diagnosis was 54.9 years (10.5), most of our patients were postmenopausal (27, 67.5%), and all but 1 were no smokers (39, 97.5%). Median body mass index (BMI) was 24.6 (4.3). As commonly observed, the vast majority of our patients exhibited a high grade (33, 82.5%), high stage (30, 75%) serous cystoadenocarcinoma at diagnosis. In a limited number of these women, one or more degrees had been diagnosed with malignancies over the previous years (7, 17.5). However, none of the familial medical histories fuelled doubts concerning BRCA defective diseases, neither these patients were genetically assessed. The performance status (PS) was on average acceptable. For the vast majority of these women, laparotomy represented the surgical treatment of choice (26, 65%).

Staining for GLUT1 was observed at the cell membrane level, in yellowish brown (figure 1a and 1b). Positive staining was observed in all the cancer cases assessed,

with strong staining being described in 29 (72.5) of them. The agreement between the two independent reviewers was 100%.

The association between circulating levels of fasting glucose and intensity of staining for GLUT1 in the 40 OvCa patients of interest is shown in table 2. Strong GLUT1 staining was inversely associated with the circulating levels of fasting glucose across glucose tertiles, whose cut off values were defined at this population level (p=0.044). The difference in distribution between weak and strong staining appeared particularly evident for patients in the lowest fasting glucose tertile (N:13), wherein the ratio between week and strong staining was 1:12.

Discussion

We herein report on the results from a restricted, though well characterized, cohort of patients with high stage, serous OvCa who were diagnosed and treated at the Regina Elena National Cancer Institute. This is an ancillary study from a larger observational study of 147 OvCa patients. In this latter cohort we first observed an inverse association between high FIGO stage at diagnosis and circulating level of fasting glucose. Our interest towards the characterization of factors involved in OvCa onset and progression, jointly with the renown prognostic relevance of stage at diagnosis in the disease of interest, prompted us to verify the biologic plausibility of the above reported association. We thus randomly extracted a subset of 40 OvCa patients for whom clinically annotated tissue samples were still available and assessed them by immunohistochemistry for GLUT1 expression. The results observed were in key with the hypothesis stated in our previous work (Vici et al. 2016). Indeed, we were able to confirm a significantly stronger GLUT1 staining in cases whose fasting glucose values fell in the lowest tertile of the distribution in this study population.

The role played by GLUT1 expression in carcinogenesis has been repeatedly investigated for ovarian and other cancers. In a quite recent study, Cai et al have observed loss of GLUT1 expression in tissue samples from non oncologic patients, while in the benign, borderline and malignant tumors the intensity of staining reported in percentages were 66, 100 and 100, respectively (Cai et al. 2014). Within this same study, the authors also found evidence of a direct association between GLUT1 expression and stage at diagnosis, which is consistent with the findings from prior studies (Kalir et al. 2002, Tsukioka et al. 2007). This is also confirmed by our results from the main study (Vici et al. 2016) and from the current analysis, when addressing distribution of cancer stage at diagnosis across tertiles of fasting glucose (supplementary table 1). Indeed, 12 out of the 13 patients from the subgroup of patients with the lowest fasting glucose levels were diagnosed with high FIGO stage tumors. The remarkable representation of high FIGO stage OvCa cases among those with intense staining for GLUT1 is also in key with a lower chance for optimal cytoreduction, as reported by Seman and co-authors based on data from 213 patients with OvCa assessed by immunohistochemistry for GLUT-1, Ki-67, and vascular endothelial growth factor (Semaan et al. 2011). More in general, results from several studies are consistent in supporting the negative prognostic and, in some cases, predictive role of GLUT1 expression in several pathologic conditions, including esophageal cancer, colorectal cancer and renal cell cancer (Egawa-Takata et al. 2010, Fonteyne et al. 2009, Lamkin et al. 2009, Martins et al. 2016, Sawayama et al. 2014, Shao et al. 2007, Tohma et al. 2005, Tsukioka et al. 2007, Wang et al. 2016).

Results from the present work may be interpreted in light of the key role played by GLUT1 in the adaption of the host organisms to the compelling changes required by carcinogenesis in terms of energy and metabolic consumption. Indeed, as the most important carrier of mammalian glucose transport across cell membranes into the cell, GLUT1 involvement has been ascertained not only under physiologic

circumstances, but also in pathologic conditions spanning from inflammation to cancer (He et al. 2007).

Our work is entirely based on the association between the results obtained from the immunostaining and the previously available data on fasting glucose levels. In the present manuscript, no referral is made to patients' important outcomes. The process of data collection for treatment outcomes including platinum sensitivity/resistance, progression free survival and overall survival, was started long ago and has now covered approximately half of the original cohort. The current study, whose limited size was dictated by its spontaneous nature, is statistically underpowered to address research questions pertinent to treatment outcomes. However, and in the full respect of the previously cited limitations, survival data seemed to provide some evidence of better outcomes in the subgroup of patients placed within the highest tertile of fasting glucose, which is also characterized by the weakest staining (supplementary figure 1). As expected, such evidence is not statistically relevant (p=0.57). In addition, the association addressed seems not linear across the fasting glucose tertiles, which may indicate the existence of a threshold to be reached for highlighting the effect of interest. This finding is somewhat consistent with our prior work on the predictive role of fasting glucose in breast cancer from our research group (Barba et al. 2012).

Novelty and feasibility represent the main strengths of this work. Although several previous studies have assessed the role of GLUT 1 in ovarian carcinogenesis, with quite consistent results, we report on a first time finding concerning the association between a biomarker of glucose metabolism expressed at the ovarian tissue level, i.e., GLUT1, and an easily and cheaply available biomarker of energy metabolism which reflects the overall systemic asset of the host organism. As a matter of fact, fasting glucose measurement is ordinarily performed in view of our patients' surgical and/or laparoscopic management, and as such, is easily amenable to be systematically recorded not only for clinical but also for research purposes. At the same time, GLUT1 assessment at the ovarian cancer tissue level is a highly standardizable

process, with overall affordable costs. In addition, from a methodological standpoint, the centralization of any procedure related to the collection, short and long term storage and assessment of both sera and tissue samples adds substantial value to our work and increase our confidence in the results obtained.

This study also has some weaknesses. The number of samples assessed is limited, although not excessively, particularly in light of relative low frequency of the disease of interest (*Ovarian cancer statistics | World Cancer Research Fund International*). As previously mentioned, the complete lack of outcome data will be soon counterbalanced by the results of the outcome analysis on the original cohort. In addition, the exclusive use of an immunohistochemical assessment in the approach to the association of interest may need integrations from different assessment levels. The impact of GLUT expression and circulating levels of fasting glucose in this patient subset and in the larger series is current under investigation in a study focused on the role microRNA in outcome prediction and interpretation in high grade serum ovarian cancer patients treated with platinum based regimens.

In conclusions, throughout the conduct of a study including data from an homogeneous case series of 40 non diabetic patients diagnosed with high FIGO stage OvCa at one single Institution, we first observed and reported on the existence of a significant inverse association between immonostaining of OvCa tissues for the glucose transporter GLUT1 and circulating levels of fasting glucose assessed at the Institutional laboratories. Although well aware of the relatively restricted size of the study population, the highly standardized procedures applied to this single institution series make us confident in the quality of our data and encourage further stepping along our research pipeline on the metabolic roots of OvCa. Pending confirmation from further and more adequately sized studies, our results may provide further support to the use of energy biomarkers in better defining patient prognosis and informing therapeutic decisions and related outcome interpretation.

Acknowledgements

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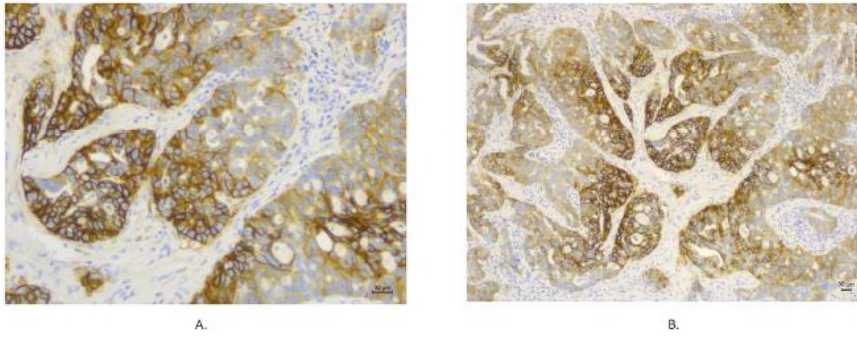
Figure 1. Photomicrographs of a case of serous ovarian cystoadenocarcinoma with intense positive staining for glucose transporter protein 1 (GLUT1). A. GLUT1 x20; B. GLUT1x10.

Table 1: Main baseline patient characteristics (N:40).

Characteristics				
[1]Age at cancer diagnosis	(mean, ±SD)		54.9	±10.5
Menopausal Status	(N, %)	*Premenopausal*	13	*32.5*
		Postmenopausal	27	*67.5*
Smoking status	(N, %)	*Yes*	1	*2.5*
		No	39	*97.5*
[2]Height at Baseline	(mean, ±SD)		159.2	±5.5
[3] Weight at Baseline	(mean, ±SD)		62.3	±11.4
[4]BMI at Baseline	(mean, ±SD)		24.6	±4.3
Grade		*G1*	1	*2.5*
		G2	6	*15.0*
		G3	33	*82.5*
[5]FIGO Stage at Cancer Diagnosis	(N, %)	*I*	5	*12.5*
		II	5	*12.5*
		III	29	*72.5*
		IV	1	*2.5*
[6]Fasting Glucose at Baseline [#]	(mean, ±SD)		95.7	±20.1
Familiarity		*Yes*	7	*17.5*
		No	33	*82.5*
[7]PS		*0*	36	*90.0*
		1	4	*10.0*
Type of surgery		[8]*LPS*	14	*35.0*
		[9]*LPT*	26	*65.0*

[1] in years, [2] in centimeters, [3] in kilograms (Kg), [4] Body Mass Index in m^2/Kg, [5] International Federation of Gynecology and Obstetrics; [6] milligrams/dl; [7] Performance Status; [8] Laparoscopy; [9] Laparotomy

Table 2: Associations between circulating levels of tertiles of fasting glucose and intensity of staining for transporter protein 1 (GLUT1) in ovarian cancer patients (N:40).

		[1]Fasting glucose			
		≤86	86-94	>94	Chi2
		N (%)	N (%)	N (%)	p-value
[2]GLUT1 SI	0-1	1 (9.1)	3 (27.3)	7 (63.6)	0.044
	2-3	12 (41.4)	10 (34.5)	7 (24.1)	

[1] Milligrams/dl; 2 Intensity of staining for GLUT was quantified using a 0 to 3 score, as it follows: no marking (0); less than 20% (1+); 20–50% (2+); and more than 50% (3+).