Archives Of Breast Cancer

DOI: 10.19187/abc.20185268-75

Epstein-Barr Virus is Associated With Aggressive Subtypes of Invasive Ductal Carcinoma of Breast (Her2+/ER- and Triple Negative) and With Nuclear Expression of NFκB p50

Ashley James Ballard[a]*

[a] Department of Cellular Pathology, The Royal Bournemouth Hospital, The Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust, Castle Lane East, Bournemouth, Dorset, United Kingdom

ARTICLE INFO

Received:
12 April 2018
Revised:
20 April 2018
Accepted:
31 April 2018

key words:
Epstein-Barr virus,
breast cancer,
estrogen receptor,
Her2 receptor,
nuclear factor κB

ABSTRACT

Background: A growing body of evidence suggests a possible role for Epstein-Barr virus (EBV) in the pathogenesis of a subset of breast cancers, with many of these studies highlighting an increased association between EBV and aggressive forms of breast carcinoma. This study aimed to further investigate this issue by assessing the possible association between EBV and the Her2+/ER- and Triple negative sub types of invasive ductal carcinoma (IDC).

Methods: An immunohistochemical marker for EBV (Epstein-Barr virus nuclear antigen 1 (EBNA1) clone E1-2.5) was applied to tissue micro array sections. The tissue micro array's contained 58 cases of Her2+/ER- IDC, 57 cases of triple negative IDC and 67 cases of luminal like IDC. Each case was scored as positive or negative for nuclear expression of EBNA1 in tumour cells using standard light microscopy. Clinical and pathological details where noted for each case, as was the nuclear expression of NFκB p50.

Results: EBV infection was apparent in 43.2% of all cases. By subtype EBV was evident in 31 (57.4%) Her2+/ER- cases, 28 (49.1%) triple negative cases, and 14 (24.1%) luminal like cases; with a significant association being noted between the Her2+/ER- and triple negative cases and EBV infection (P 0.001). This association was primarily linked with ER negativity, Her2 status showed no significant association with EBV infection. There were no significant associations with other clinical and pathological characteristics. Of the 53 cases demonstrating NFκB p50 nuclear staining, 37 (69.8%) were also infected by EBV ($P<0.001$).

Conclusion: This study provides evidence that EBV is associated with aggressive subtypes of IDC (Her2+/ER- and triple negative) as well as providing evidence for a link between EBV and NFκB p50 nuclear expression, although the nature of these associations remains unclear.

Introduction

Epstein-Barr virus is known to be associated with a number of epithelial tumours, including nasopharyngeal carcinoma[1] and a subset of gastric carcinomas.[2] Over the past 20 years a growing body of evidence has also suggested a role for EBV in a subset of sporadic breast cancers.[3-13] The first of these studies identifying EBV with breast cancer in 1995 failed to identify it with any particular histological type.[12,13] Following on from this Fina *et al*.[9] conducted a large multi centric study in 2001, this appeared to confirm both the association of EBV with breast cancer and the lack of any correlation with clinical or pathological characteristics (age, histological grade, tumour size, nodal status). However, more recent investigations have highlighted associations between

Address for correspondence:
Ashley James Ballard, M. D.
Address: Department of Cellular pathology, The Royal Bournemouth and Christchurch Hospitals, Castle Lane East , Bournemouth, Dorset, BH7 7DW, United Kingdom
Email: Ashley.ballard@rbch.nhs.uk
ashleyballard@hotmail.co.uk

EBV and high histological grade, nodal involvement, and young age of onset,[3,5-7] while a meta-analysis published in 2012 appeared to demonstrate an association between EBV infection and elevated breast cancer risk.[14] There is also some evidence to suggest a link between EBV and ER status. Using immunohistochemistry (IHC) to detect Epstein-Barr virus nuclear antigen 1 (EBNA1) Murray et al[5] found a strong association between the 2B4-1 EBNA1 clone and ER negative tumours, although this was in the absence of PCR detectable EBV genetic material. Following on from this, two further studies using polymerase chain reaction (PCR) based methods[3, 4] demonstrated a clear relationship between EBV and ER negative breast tumours; although some investigators[6, 7] have failed to demonstrate significant associations between EBV and ER negative tumours this was largely due to small sample sizes, overall the data appears to be in favour of an association. However, as yet no clear molecular mechanism has been demonstrated to explain this. The relationship between EBV and Her2 status is unclear at present; there is evidence of Her2 overexpression in *in-vitro* models using EBV infected breast cancer cell lines.[15] However, of the three studies to investigate this issue in human breast tissue, the first failed to demonstrate any association between EBV and Her2,[4] while the second demonstrated a weak association between EBV and Her2 gene amplification.[3] In the third study by Glenn *et al.* in 2012[6] all the Her2 expressing tumours were also found to be infected by EBV, although, due to the small sample size this was not considered significant.

From the evidence set out above it appears that breast tumours infected by EBV have a more aggressive nature, although this is not yet conclusive, and the manner of the relationship remains uncertain. Following on from the studies outlined above, the primary aim of this investigation was to further examine the relationships between EBV infection and the aggressive triple negative and Her2+/ER- subtypes of invasive ductal carcinoma (defined by receptor status). It was hypothesised that both of these subtypes would demonstrate significantly higher levels of EBV infection compared to the luminal like group (ER+/Her2- and ER+/Her2+/PR+). However, considering the evidence outlined above it was expected that ER status would have a greater impact than Her2 status. In order to test this IHC was used to examine expression levels of EBNA1 in 182 cases of breast cancer in tissue micro arrays (TMA's), the use of IHC would allow EBV infection to be localised to tumour cells. The clinical and pathological characteristics of the tumours were also assessed to determine if there was a relationship with EBV infection; these included age, histological grade, tumour size, nodal involvement and Ki67 status. In addition to this NFκB nuclear expression was also assessed, as this transcription factor is known to be associated with EBV in gastric and nasopharyngeal carcinoma.[16-18]

Method

Tissue specimens

The study used TMA's (BR1503b, BR1504, BR1505, US BIOMAX) consisting of 410 cores of invasive ductal carcinoma in total, there were 2 cores from each case (total of 205 cases of IDC). The cases were grouped according to subtype of IDC (as defined by ER/PR/Her2 receptor expression). Cases that did not match the receptor expression patterns were discounted from the study.

ER/PR and Her2 had been scored previously (information provided by US BIOMAX), ER/PR were considered positive if more than 1% of tumour cells were positive with an intensity of 1 (week) or stronger (>2 using the Allred scoring method). A Her2 score of 3+ was considered positive, a score of 0/1+ was considered negative. For the purposes of this study a score of 2+ was considered equivocal and the case was discounted (no FISH data available).

There were 58 cases of Her2+/ER- IDC (ER-, PR+/-, Her2+), 57 cases of triple negative IDC (ER-, PR-, Her2-) and 67 cases of luminal-like IDC (ER+, PR+/-, Her2- and ER+, PR+, Her2+). The proliferation level of each case had also been determined by US BIOMAX prior to the study using Ki67. The proliferation level was considered low if ≤20% of tumour cells expressed Ki67 or high if >20% of tumour cells expressed Ki67. Information about the clinical and pathological characteristics of the cases (Age, Grade, Size, and Nodal involvement) was provided by US BIOMAX, this data can be seen in Table 1. NFκB p50 nuclear staining was assessed by IHC within the testing laboratory using the method outlined below. TMA's were stained using an EBNA1 monoclonal antibody, and were visualised according to the method outlined below. If the two cores from a single case did not display the same staining characteristics the result was considered equivocal and the case was discounted from the study.

Antibodies and control material

Commercially available control material was used that was known to stain strongly for EBV (245S, CELLMARQUE), and NFκB (326S, CELLMARQUE). To assess NFκB nuclear staining the NFκB p105/p50 antibody was used (clone 5D10, mouse monoclonal IgG1, ABCAM), diluted 1:200. To assess EBV infection the EBNA1 antibody was used (clone E1-2.5, mouse monoclonal IgG1, ABCAM), diluted 1:2000. Both antibodies were diluted using common antibody diluent (HK156-5KE, BIOGENIX).

Immunohistochemistry (IHC)

The tissue micro arrays were de waxed in xylene taken through graded alcohols and re hydrated. TMA's were then immersed in 10% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity and retrieved according to manufacturer's guidelines (heat mediated using the DAKO Pascal chamber and pH6.0 buffer [RE7113, NOVACASTRA]). After rinsing in wash buffer (HK583-5KE, BIOGENIX), the TMA's were stained using a BIOGENIX i6000 with appropriate antibodies. Commercial control tissue was included with each staining run, the negative control was stained using a mouse IgG1 negative control (X0931 mouse monoclonal IgG1, DAKO). The antibodies were visualised using the SuperSensitive Polymer HRP (diaminobenzidine) kit (QD430-XAKE, BIOGENIX). Sections were then counterstained using Mayer's haemalum, differentiated in 0.25% acid alcohol, blued using ammonium water and were dehydrated in alcohol, cleared in xylene and coverslipped. Sections were viewed and graded (positive/negative) using standard light microscopy, only nuclear staining within breast tumour cells was considered positive.

Statistical analysis

Statistical analysis was carried out using a statistical package for social sciences (SPSS) version 20 (PASW, IBM Corp. USA 2011). Associations between categorical data were assessed using the Chi-square test (χ^2) or Fisher's exact test where appropriate. The Mantel-Haenszel test was used when appropriate to assess the associations between categorical data where a third variable acted as a possible confounding factor to the two variables of primary interest. Using the Bonferroni correction, a P value <0.005 was considered significant. Risk analysis was used to determine odds ratios (OR) and confidence intervals (CI).

Results

EBV (EBNA-1 nuclear positivity) results

182 cases of invasive ductal carcinoma were assessed using IHC for the presence of EBV (EBNA1) in the nuclei of tumour cells. 13 cases were either unreadable or displayed equivocal staining and were discounted from the study. 169 cases remained that were eligible consisting of 58 cases of luminal like IDC (34.3%), 54 cases of Her2+/ER- IDC (32.0%), and 57 cases of triple negative IDC (33.7%). EBNA1 staining patterns broken down according to the clinical and pathological characteristics of the cases can be seen in Table 1.

EBNA1 staining was evident in the tumour cells of 73 cases (43.2%), with a statistical power greater than 0.80; there was no evidence of EBNA1 staining in surrounding tissue or infiltrating lymphocytes. A significant association can be seen between EBV infection and the three IDC subtypes investigated [$\chi^2(2) = 13.85$, P 0.001]; with EBNA1 positive breast tumour cells present in 31 (57.4%) of the Her2+/ER- cases and 28 (49.1%) of the triple negative cases, but only 14 (24.1%) of the luminal like cases. A three way analysis of the IDC subtypes association with EBV infection showed that the most significant difference was between the Her2+/ER- and luminal like subtypes [$\chi^2(1) = 12.88$, P<0.001], with the odds ratio indicating that EBV infection was 4.24 times more likely in the Her2+/ER- subtype (95% CI, 1.89-9.50). The difference between the triple negative and luminal like sub types was also shown to be (borderline) significant [$\chi^2(1) = 7.74$, P 0.005], with the odds ratio indicating that EBV infection was 3.03 times more likely in the triple negative subtype (95% CI, 1.37-6.71). There was no significant difference between the Her2+/ER- and triple negative sub types.

Receptor, clinical and pathological staining patterns

A significant association was shown between ER negativity and EBV infection [$\chi^2(1) = 13.07$, P <0.001], with 53.2% of ER negative tumours shown to be EBNA1 positive, compared to only 24.1% of ER positive tumours. Based on the odds ratio, EBV infection is 3.57 times more likely in ER negative tumours (95% CI 1.76-7.24). It was noted that both the Her2+ and PR- groups overlapped significantly with the ER- group (54 Her2+ cases in the ER- group, 108 PR- cases in the ER- group), therefore the Mantel-Haenszel test was used to adjust for ER status when assessing PR status and Her2 status. Adjusting for ER status no significant association was found between PR status and EBV infection [Mantel-Haenszel $\chi^2(1) = 0.03$, P 0.863], similarly no association was evident between Her2 status and EBV infection [Mantel-Haenszel $\chi^2(1) = 0.69$, P 0.405].

Adjusting for tumour subtype a significant association was shown between NFκB p50 nuclear staining and EBV infection [Mantel-Haenszel $\chi^2(1) = 15.80$, P <0.001], with 69.8% of tumour cells demonstrating NFκB p50 nuclear staining also shown to be EBNA1 positive. Based on the odds ratio EBV infection is 4.11 times more likely in those tumours demonstrating nuclear expression of NFκB p50 (95% CI 2.03-8.35). However, there were no significant relationships between EBNA1 positivity and age at diagnosis, histological grade, tumour size, nodal involvement or Ki67 status (see Table 1).

Discussion

Many of the investigations to date have utilised PCR or EBV encoded small RNAs (EBERs) *in situ* hybridisation as the primary detection methods. However, PCR has been criticised for detecting EBV DNA in infiltrating lymphocytes (laser capture micro dissection used in later studies should have

Table 1. EBV (EBNA1) staining according to the clinical and pathological characteristics (by case)

Characteristics		N (cases)	(EBNA1) EBV+ (%)	(EBNA1) EBV- (%)	P value
All		169	73 (43.2)	96 (56.8)	
Age	<50	80	30 (37.5)	50 (62.5)	0.10
	≥50	89	43 (48.3)	46 (51.7)	
Histological grade	Grade I	16	6 (37.5)	10 (62.5)	0.34
	Grade II	124	57 (46.0)	67 (54.0)	
	Grade III	23	7 (30.4)	16 (69.6)	
Tumour size	<2 cm	10	4 (40.0)	6 (60.0)	0.56
	≥2 cm	158	68 (43.0)	90 (57.0)	
Nodal involvement	N-	139	58 (41.7)	81 (58.3)	0.27
	N+	30	15 (50.0)	15 (50.0)	
Ki67 status	Low	91	38 (41.8)	53 (58.2)	0.40
	High	78	35 (55.1)	43 (44.9)	
NFκB p50 status	Negative	116	36 (31.0)	80 (69.0)	**<0.001**
	Positive	53	37 (69.8)	16 (30.2)	
Estrogen receptor	Positive	58	14 (24.1)	44 (75.9)	**<0.001**
	Negative	111	59 (53.2)	52 (46.8)	
Progesterone receptor	Positive	47	12 (25.5)	35 (74.5)	0.86
	Negative	122	61 (50.0)	61 (50.0)	
Her2 status	Positive	60	33 (55.0)	27 (45.0)	0.40
	Negative	109	40 (36.7)	69 (63.3)	
Subtype	Luminal like	58	14 (24.1)	44 (75.9)	**0.001**
	Her2+/ER-	54	31 (57.4)	23 (42.6)	
	Triple negative	57	28 (49.1)	29 (50.9)	

alleviated this issue), and reports have indicated an EBER negative form of EBV infection could be present in breast tumour cell.[3, 14, 19] A number of investigations have also used immunohistochemistry as either the primary or secondary detection system, allowing identification of cell type (differentiating infected tumour cells from infiltrating lymphocytes). However, a number of different markers (EBNA1, EBNA2, latent membrane protein 1 (LMP1), and LMP2A) have been used in various studies leading to conflicting results. Recent work appears to indicate that EBNA1 and LMP2A are both positive while LMP1 and EBERs are negative in breast cancer.[19] The reliability of some of the earlier IHC studies must also be questioned due to technical concerns, an early showed strong staining of tumour cells using the 2B4-1 EBNA1 clone while failing to demonstrate the presence of EBV genetic material within the same cells,[5] this clone has subsequently been found to cross react with the MAGE4 protein, a testis tumour antigen also expressed in some breast cancers.[20] The immunohistochemistry method has been used in the present study, allowing the investigator to ensure EBV was localised to breast tumour cells. The EBNA1 monoclonal antibody (clone E1-2.5) was employed to detect EBV, as EBNA1 is thought to be expressed though all the latent phases of viral infection.[4] The E1-2.5 clone was chosen as it has been shown to be more reliablethan the 2B4 clone.[7,20]

Previous investigations have demonstrated EBV positivity ranging from 21-63% with a mean of 35.5% (SD=10.9) when considering all forms of investigation, when considering only IHC staining using EBNA1 the range is 25-55% and the mean is 35.4% (SD=10.3).[3-13, 19, 21] Although the results of the present study are slightly above the mean (43.2%), they are well within the range and fall within 1SD. The higher level of positivity in the current study may be due to the nature of the sample. Previous investigations used samples representative of the population norm in terms of receptor expression; due to the nature of this investigation the sample contained more ER- and Her2+ cases than would normally be expected. However, due to the nature of the investigation (using TMA's), it was not possible to accurately classify lymphocytic infiltration of the tumour tissue. This should be considered when viewing these results.

Gene expression studies have identified five primary subtypes of breast cancer (invasive ductal carcinoma, no special type), Luminal A, Luminal B, Normal like, Basal like and Her2 positive.[22] However, from an oncologist's point of view breast cancer patients fall in to one of three groups, ER positive, Her2 positive and triple negative,[23] as these groups represent distinct prognostic outcomes.[24] A

strong association was demonstrated between EBV infection and tumour subtype (P=0.001), with EBV infection shown to be more prevalent in the Her2+/ER- and triple negative subtypes of invasive ductal carcinoma. This points towards a possible role for EBV in the pathogenesis of these breast cancer subtypes, both of which are typically high grade and aggressive.[25] This study directly investigates the association between EBV infection and the high risk IDC subtypes (Her2+/ER- and Triple negative) in an organised manner, and backs up the work of previous authors associating EBV infection with aggressive (ER-) breast tumours.[3,4]

To gain a greater understanding of the nature of this relationship receptor (ER, PR, Her2) receptor expression profiles were examined (although data on ER, PR, and Her2 clones used for this was not available), with Mantel-Haenszel analysis being used to adjust for ER status. As can be seen from the results ER negativity appears to be the dominant factor in the association between EBV and the aggressive breast cancer sub types, with the odds ratio suggesting that EBV infection is 3.57 times more likely in ER negative tumours (P <0.001). However, the nature of the relationship remains unclear, as no molecular mechanism has been outlined as yet to explain the association. It may be that the higher proportion of ER negative cells in the basal layer makes these cells more susceptible to the transfer of EBV infection from infiltrating lymphocytes, although the recent report by Khan *et al.*[26] failed to find a correlation between EBV infected infiltrating lymphocytes and ER negative breast tumours. Further studies are needed to examine this issue, as the small size of the cores in the TMA's limited the ability of the investigator to fully screen for infiltrating lymphocytes in the present study. No association was evident between the EBV infected tumour cells and PR receptor expression, confirming the findings of previous authors.[3,4,7] As outlined in the introduction the evidence for an association between EBV infection and Her2 expression is mixed, it was reported by Lin *et al.*[15] that infection of breast cancer cell lines by EBV leads to activation of the Her2/Her3 signalling axis and elevated Her2 expression through the action of the BARF0 gene product. EBV has also been shown to induce EGFR expression in cervical carcinoma cell cultures through the action of NFκB p50,[27] and recent evidence suggests that the ERBB2 gene is amplified in a subset of EBV linked gastric carcinomas.[28] Taken together this evidence suggests that an intimate relationship may exist between EBV and the Her family of receptors in a range of carcinomas. However, in this current study no association was observed between EBV infection and Her2 overexpression. This is broadly in agreement with previous studies that have examined this relationship which found either weak associations,[3] or a lack of any significant association.[4,6]

The clinical and pathological profiles (patient age, histological grade, tumour size, and nodal involvement) of the EBV positive and EBV negative tumours were investigated as relationships between these factors and EBV have been noted in previous studies. An early study demonstrated a significant association between EBNA1 staining (using the 2B4-1 clone) and tumour size, tumour grade and nodal involvement; presence of the EBNA1 protein was found to be associated primarily with grade 3 tumours greater than 50 mm with more than 3 lymph nodes positive for metastases.[5] However, this study failed to demonstrate the presence of the EBV genome using PCR, and the reliability of the 2B4-1 clone must also be questioned as outlined earlier in the discussion. A further study in 2008 showed support for the association between EBV infection and high grade node positive tumours,[7] although no significant association was evident between EBV infection and tumour size. Another study in 2011[3] demonstrated an association between EBV positivity and high tumour grade, however, no other clinical or pathological factors were significant (excluding receptor expression status). A final study by Glenn et al. in 2012[6] indicated that EBV was associated with a younger age of diagnosis. In the present study no associations were evident between EBV infection of breast tumours and age, histological grade, tumour size or nodal involvement; this is in accordance with the majority of previous investigations,[4,8,10,11] including a large multi centric study by Fina *et al.* in 2001.[9] The scores used to grade the IDC sub-types in the TMA's used were not available, so it was not possible to assess if EBV infection was associated with different scores for nuclear pleomorphism, or tubule formation. However, the relationship between EBV positivity and Ki67 status was assessed; Ki67 measures the proliferation activity of tumours and is becoming increasingly important in breast cancer prognostic tests such as IHC4+C.[29, 30] However, no significant association was evident. None of the previous studies investigating EBV and breast cancer have observed Ki67 status.

NFκB is a rapid acting primary transcription factor found in most cell types that acts as a master regulator of cellular responses to stress; it serves as a primary means of relaying signals from the extracellular environment to the nucleus in order to initiate a genetic program. Aberrant expression of NFκB has been associated with a number of cancers.[31] In breast cancer it has been found to be correlated with ER/PR negativity, and is associated with the Her2 positive and basal like subtypes;[32, 33] NFκB p50 and p65 are both elevated in these cancer types, however, levels of NFκB p50 have been shown to be significantly higher than p65. NFκB has also been strongly linked to radiotherapy and anti estrogen therapy resistant forms of breast cancer.[31,34]

Increased NFκB expression is normally the result of physiological changes leading to its activation. In the case of breast cancer, Her2 positive and basal like tumours have a solid growth pattern that leads to increased hypoxia towards the centre of the tumour mass, NFκB is one of the transcription factors governing cellular responses to hypoxia.[33] EBV has been linked to NFκB in both gastric and nasopharyngeal carcinoma.[16-18] In addition, a series of investigations have demonstrated EGFR receptor up regulation due to the activation of EGFR promoters by NFκB p50/p50/Bcl-3 complexes,[27, 35] this was through the action of the LMP1 C-terminal activating region 1(CTAR1). A further study in the same series indicated that this LMP1-CTAR1 mediated up regulation of EGFR by p50/p50/Bcl3 complexes is not dependent on the standard NFκB pathway,[36] indicating a distinct role for EBV in tumour genesis. The results from the present study indicate that there is an unexpectedly strong association between EBV infection and nuclear expression of NFκB p50 in cases of invasive ductal carcinoma ($P < 0.001$); the Mantel-Haenszel common odds ratio indicated that NFκB p50 nuclear expression was 4.11 times more likely in tumours infected with EBV (after adjusting for tumour sub-type). However, the nature of this association remains unclear; from the evidence outlined earlier in the discussion it is unlikely that EBV LMP1 is interacting with NFκB, it is possible that LMP2A interacts with NFκB in EBV linked breast cancer as is the case in EBV linked gastric carcinoma.[17, 37] It seems clear from the results of this investigation as well as from previously published data that EBV is not associated with Her2 in breast cancer;[4] therefore, it is possible that LMP2A interacts with the NFκB pathway to confer resistance to apoptotic stimulus in cases of EBV linked breast cancer, as is the case in EBV linked gastric carcinoma.[17] Further studies should be carried out to confirm these initial findings, and investigate the association between EBV and NFκB in breast cancer.

The primary aim of this study was to investigate a possible association between EBV infection and aggressive sub types of invasive ductal carcinoma. The data clearly indicates an association, with EBV infection significantly more prevalent in the triple negative (49.1%) and Her2+/ER- (57.4%) sub types compared to the luminal like sub type (24.1%). It was further demonstrated that a significant association is present between EBV infection and ER negativity, however, no significant associations exist between EBV infection and either PR status or Her2 status. The nature of the relationship between ER negativity and EBV infection remains unclear, with additional investigations needed to determine if ER negativity is involved in the aetiology of a possible sub set of EBV linked aggressive breast tumours, due to the limitations inherent with commercial TMA's further work may be required to confirm these results. A surprisingly strong association was also noted between EBV infection and nuclear expression of NFκB p50 (independent of tumour sub-type), although the nature of this relationship remains unclear, and further studies are necessary to confirm these results and to determine how EBV interacts with NFκB. This study failed to find evidence of associations between EBV infection and other clinical and pathological characteristics (age, histological grade, nodal involvement, tumour size and Ki67 status).

Conflict of Interest

The author has no conflict of interest in relation to this article.

References

1. Burgos JS. Involvement of the Epstein-Barr virus in the nasopharyngeal carcinoma pathogenesis. Medical oncology. 2005;22(2):113-21.
2. Fukayama M, Ushiku T. Epstein-Barr virus-associated gastric carcinoma. Pathology, research and practice. 2011;207(9):529-37.
3. Mazouni C, Fina F, Romain S, Ouafik L, Bonnier P, Brandone JM, *et al.* Epstein-Barr virus as a marker of biological aggressiveness in breast cancer. British journal of cancer. 2011;104(2): 332-7.
4. Hachana M, Amara K, Ziadi S, Romdhane E, Gacem RB, Trimeche M. Investigation of Epstein-Barr virus in breast carcinomas in Tunisia. Pathology, research and practice. 2011;207(11):695-700.
5. Murray PG, Lissauer D, Junying J, Davies G, Moore S, Bell A, *et al.* Reactivity with A monoclonal antibody to Epstein-Barr virus (EBV) nuclear antigen 1 defines a subset of aggressive breast cancers in the absence of the EBV genome. Cancer research. 2003;63(9): 2338-43.
6. Glenn WK, Heng B, Delprado W, Iacopetta B, Whitaker NJ, Lawson JS. Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. PloS one. 2012;7(11):e48788.
7. Fawzy S, Sallam M, Awad NM. Detection of Epstein-Barr virus in breast carcinoma in Egyptian women. Clinical biochemistry. 2008;41(7-8):486-92.
8. Joshi D, Quadri M, Gangane N, Joshi R, Gangane N. Association of Epstein Barr virus infection (EBV) with breast cancer in rural Indian women. PloS one. 2009;4(12):e8180.
9. Fina F, Romain S, Ouafik L, Palmari J, Ben Ayed F, Benharkat S, *et al.* Frequency and genome load of Epstein-Barr virus in 509 breast cancers from different geographical areas. British journal of cancer. 2001;84(6):783-90.

10. Preciado MV, Chabay PA, De Matteo EN, Gonzalez P, Grinstein S, Actis A, *et al.* Epstein-Barr virus in breast carcinoma in Argentina. Archives of pathology & laboratory medicine. 2005;129(3):377-81.
11. Zekri AR, Bahnassy AA, Mohamed WS, El-Kassem FA, El-Khalidi SJ, Hafez MM, *et al.* Epstein-Barr virus and breast cancer: epidemiological and molecular study on Egyptian and Iraqi women. Journal of the Egyptian National Cancer Institute. 2012;24(3):123-31.
12. Labrecque LG, Barnes DM, Fentiman IS, Griffin BE. Epstein-Barr virus in epithelial cell tumors: a breast cancer study. Cancer research. 1995;55(1):39-45.
13. Luqmani Y, Shousha S. Presence of epstein-barr-virus in breast-carcinoma. International journal of oncology. 1995;6(4):899-903.
14. Huo Q, Zhang N, Yang Q. Epstein-Barr virus infection and sporadic breast cancer risk: a meta-analysis. PloS one. 2012;7(2):e31656.
15. Lin JH, Tsai CH, Chu JS, Chen JY, Takada K, Shew JY. Dysregulation of HER2/HER3 signaling axis in Epstein-Barr virus-infected breast carcinoma cells. Journal of virology. 2007;81(11):5705-13.
16. Tang W, Morgan DR, Meyers MO, Dominguez RL, Martinez E, Kakudo K, *et al.* Epstein-barr virus infected gastric adenocarcinoma expresses latent and lytic viral transcripts and has a distinct human gene expression profile. Infectious agents and cancer. 2012;7(1):21.
17. Fukayama M, Hino R, Uozaki H. Epstein-Barr virus and gastric carcinoma: virus-host interactions leading to carcinoma. Cancer science. 2008;99(9):1726-33.
18. Ren Q, Sato H, Murono S, Furukawa M, Yoshizaki T. Epstein-Barr virus (EBV) latent membrane protein 1 induces interleukin-8 through the nuclear factor-kappa B signaling pathway in EBV-infected nasopharyngeal carcinoma cell line. The Laryngoscope. 2004;114(5):855-9.
19. Lorenzetti MA, De Matteo E, Gass H, Martinez Vazquez P, Lara J, Gonzalez P, *et al.* Characterization of Epstein Barr virus latency pattern in Argentine breast carcinoma. PloS one. 2010;5(10):e13603.
20. Murray PG. Epstein-Barr virus in breast cancer: artefact or aetiological agent? The Journal of pathology. 2006;209(4):427-9.
21. Xue SA, Lampert IA, Haldane JS, Bridger JE, Griffin BE. Epstein-Barr virus gene expression in human breast cancer: protagonist or passenger? British journal of cancer. 2003;89(1):113-9.
22. Dolle JM, Daling JR, White E, Brinton LA, Doody DR, Porter PL, *et al.* Risk factors for triple-negative breast cancer in women under the age of 45 years. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(4):1157-66.
23. Reis-Filho JS, Tutt AN. Triple negative tumours: a critical review. Histopathology. 2008;52(1):10818.
24. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, *et al.* The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13(8):2329-34.
25. Peppercorn J, Perou CM, Carey LA. Molecular subtypes in breast cancer evaluation and management: divide and conquer. Cancer investigation. 2008;26(1):1-10.
26. Khan G, Philip PS, Al Ashari M, Houcinat Y, Daoud S. Localization of Epstein-Barr virus to infiltrating lymphocytes in breast carcinomas and not malignant cells. Experimental and molecular pathology. 2011;91(1):466-70.
27. Thornburg NJ, Raab-Traub N. Induction of epidermal growth factor receptor expression by Epstein-Barr virus latent membrane protein 1 C-terminal-activating region 1 is mediated by NF-kappaB p50 homodimer/Bcl-3 complexes. Journal of virology. 2007;81(23):12954-61.
28. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014; 513(7517):202-9.
29. Barton S, Zabaglo L, A'Hern R, Turner N, Ferguson T, O'Neill S, *et al.* Assessment of the contribution of the IHC4+C score to decision making in clinical practice in early breast cancer. British journal of cancer. 2012;106(11):1760-5.
30. Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, *et al.* Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29(32):4273-8.
31. Ahmed KM, Cao N, Li JJ. HER-2 and NF-kappaB as the targets for therapy-resistant breast cancer. Anticancer research. 2006;26(6B):4235-43.
32. Shapochka DO, Zaletok SP, Gnidyuk MI. Relationship between NF-kappaB, ER, PR, Her2/neu, Ki67, p53 expression in human breast cancer. Experimental oncology. 2012;34(4):35863.
33. Biswas DK, Iglehart JD. Linkage between EGFR family receptors and nuclear factor kappaB (NF-kappaB) signaling in breast cancer. Journal of cellular physiology. 2006;209(3):645-52.
34. Yde CW, Emdal KB, Guerra B, Lykkesfeldt AE. NFkappaB signaling is important for growth of antiestrogen resistant breast cancer cells. Breast

cancer research and treatment. 2012;135(1):67-78.

35. Kung CP, Raab-Traub N. Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor through effects on Bcl-3 and STAT3. Journal of virology. 2008;82(11):5486-93.
36. Kung CP, Raab-Traub N. Epstein-Barr virus latent membrane protein 1 modulates distinctive NF- kappaB pathways through C-terminus-activating region 1 to regulate epidermal growth factor receptor expression. Journal of virology. 2010;84(13):6605-14.
37. HinoR, Uozaki H, Inoue Y, Shintani Y, Ushiku T, Sakatani T, *et al.* Survival advantage of EBV-associated gastric carcinoma: survivin up-regulation by viral latent membrane protein 2A. Cancer research. 2008;68(5):1427-35.