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FANCD2 and *BRCA1* have differential expression among the FA-BRCA genes in primary breast cancer

Expressão diferencial de *FANCD2* e *BRCA1* no câncer de mama primário

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Brazilian Journal of health Review

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ABSTRACT
The molecular pathways of DNA repair in tumors may play a role in tailoring patient therapy. The Fanconi anemia DNA repair pathway operates in the repair of DNA interstrand crosslink induced by several chemotherapeutic drugs. In this study we evaluated the expression of Fanconi anemia DNA repair genes (*FANCA*, *C*, *D2*, *F*, *BRCA1* and *PALB2)* in 46 primary breast tumors and ten non-compromised breast samples, by Real-Time Quantitative Reverse Transcription PCR, and to correlated gene expression with breast cancer subtypes and clinico-pathological parameters. Tumor samples were classified in subtypes based on immunohistochemistry markers, and clinico-pathological parameters were obtained from the medical reports. *FANCD2* was twice more expressed in tumors than in the non-compromised group (p= 0.02). *BRCA1* showed a differential expression in the luminal group, three times less expressed in Luminal-B than in Luminal-A group (p= 0.01). In conclusion, the higher level of expression of *FANCD2* in tumors may indicate activation of the Fanconi anemia DNA repair pathway, which has been implicated in breast carcinogenesis and in chemotherapeutic resistance. The loss of *BRCA1* expression in the Luminal-B group may indicate that the use of cisplatin-based neo/adjuvant therapies is preferable, and that the use of taxol-based therapies should be avoided due to the risk of drug resistance.

Keywords: DNA repair, *FANCD2, BRCA1*, gene expression, breast cancer.

RESUMO
As vias moleculares de reparo do DNA em tumores podem desempenhar um papel na adaptação da terapia do paciente. A via de reparo do DNA da anemia de Fanconi opera no reparo da reticulação entre cadeias de DNA induzida por várias drogas quimioterapêuticas. Neste estudo, avaliamos a expressão dos genes de reparo do DNA da anemia de Fanconi (*FANCA, C, D2, F, BRCA1 e PALB2*) em 46 tumores primários de mama e dez amostras de

Brazilian Journal of health Review

mama não comprometidas, por PCR de transcrição reversa quantitativa em tempo real, e para expressão gênica correlacionada com subtipos de câncer de mama e parâmetros clínico-patológicos. As amostras de tumor foram classificadas em subtipos com base em marcadores imuno-histoquímicos e os parâmetros clínico-patológicos foram obtidos nos relatórios médicos. O FANCD2 foi duas vezes mais expresso em tumores do que no grupo não comprometido (p = 0,02). O *BRCA1* mostrou uma expressão diferencial no grupo luminal, três vezes menos expresso no Luminal-B do que no grupo Luminal-A (p = 0,01). Em conclusão, o maior nível de expressão de *FANCD2* em tumores pode indicar a ativação da via de reparo do DNA da anemia de Fanconi, que tem sido implicada na carcinogênese da mama e na resistência quimioterapêutica. A perda da expressão de *BRCA1* no grupo Luminal-B pode indicar que o uso de terapias neo / adjuvantes à base de cisplatina é preferível e que o uso de terapias à base de taxol deve ser evitado devido ao risco de resistência aos medicamentos.

Palavras-chave: reparo de DNA, *FANCD2*, *BRCA1*, expressão genética, câncer de mama.

1 INTRODUCTION

Breast cancer is the most common type of cancer, and the leading cause of cancer death in women worldwide (Torre et al., 2015; Bray et al., 2018) and also in Brazil, with an estimated 61 cases for every 100 thousand inhabitants (Inca, 2020). Among the genetic alterations involved in the development of breast cancer, lack of expression of tumor suppressor genes (TSG) is of great importance, because they are involved in cell cycle control and DNA damage repair pathways. Some known causes for the inactivation of TSG are deletions, allelic imbalances, mutations and gene promoter hypermethylation (Kim et al., 2011).

Expression analyses of DNA repair genes, such as the Fanconi Anemia DNA repair pathway (*FA-BRCA*), often result in clinically relevant data, highlighting mechanisms related to tumor progression and anticipating drug response. So, this information can be used to develop appropriate therapeutic strategies. Upregulation of these genes may suggest the use of chemotherapeutic drugs acting in another pathway, preventing the repair of the desirable DNA lesions. Moreover, the downregulation when comparing with non-tumor tissues, shed light in one of the mechanisms contributing to tumor instability, which may be useful in developing new molecular therapies that are more specific and efficient with lower side effects (Altieri et al., 2008).

Twenty-two (22) *FA-BRCA* genes are known: *FANCA-C, D1, D2, E-G, I, J, L-V* and *W* (Nepal et al., 2017; Nalepa and Clapp, 2018). These genes are related to homologous recombination (HR) process, and act specifically during the repair of DNA interstrand

crosslink (ICL), which are a consequence of agents that may or may not generate large structural distortions in DNA, such as mitomycin C (MMC) and cisplatin (Noll et al., 2006; Smeaton et al., 2008). ICL that causes DNA distortions are recognizable by the DNA repair machinery, however the detection of non-distorting ICL depends on genomic transactions that occur in their vicinity, once they act as barriers to processes that require translocation along the DNA (Jones et al., 2012).

FANCA-C, E-G, L, M and T proteins, and the accessory components FAAP20, 24, 100 and MHF1/2, form the core-complex, which recognizes DNA lesions through FANCM and is required to monoubiquitylation of the FANCD2-FANCI heterodimer. Once the FANCD2-FANCI heterodimer becomes monoubiquitylated by FANCL (RING-finger containing E3 ubiquitin ligase) and FANCT (E2 ubiquitin-conjugating enzyme, UBE2T), FAN1 (FANCD2 associated nuclease) binds and localizes the heterodimer with the downstream effectors FANCN (PALB2), J (BRIP1), O (RAD51C), D1 (BRCA2), P (SLX4), Q (ERCC4), R (RAD51), S (BRCA1), U (XRCC2) and V (REV7/MAD2L2) (Doane et al., 2006; Nalepa and Clapp, 2018).

Patients lacking FANCS and D1 proteins (BRCA1/2) are more sensitive to cross-linking agents. BRCA2 act as a regulator of RAD51, an essential protein for HR repair (Deans and West, 2011). Although BRCA1 and BRCA2 are mainly involved in hereditary breast and ovarian cancer (Dorsman et al., 2007; Castralli and Bayer, 2019), they have also been implicated in sporadic breast cancer (Durkin and Glover, 2007; Ganzinelli et al., 2011).

The present study aims to evaluate the expression profile of *FA-BRCA* genes in a well characterized group of primary invasive breast tumors comparing to a group of non-compromised (NC) tissues from the contralateral breast, and to correlate gene expression with immunohistochemical (IHC) breast cancer subtypes and clinico-pathological parameters.

2 MATERIALS AND METHODS

Experimental samples: Forty-six (46) samples from primary breast tumors were obtained from 46 patients undergoing partial or complete mastectomies at *Hospital Nossa Senhora das Graças* and *Hospital das Clínicas,* Curitiba, Paraná, Brazil. The NC tissues were obtained from the contralateral breast from ten (10) patients who suffered mastectomy and mammoplasty simultaneously for breast symmetrization. Tumor and NC samples were conserved in *RNAlater*® (*Ambion Inc. Applied Biosystems*) immediately after surgery and

were stored at 4 °C prior to RNA isolation. Clinico-pathological data were obtained from the pathology records of the hospitals (Table 1). The parameters analyzed were age at diagnosis, histological subtype, IHC subtype, grade, tumor size, estrogen receptor (ER), progesterone receptor (PgR) and HER2 (ERBB2) amplification. The patients had no family history of breast or ovarian cancer. The average age of patients was 59.3 ± 16.3 years and ranged from 31 to 97 years old. Patients had received neither neoadjuvant chemotherapy nor radiation. Informed consent was obtained from all participants. The local Ethics Committee approved the study.

The IHC breast cancer subtype classification was performed based on the 13th St. Gallen Guideline (Goldhirsch et al., 2013), which defines breast tumors based on IHC staining of ER, PgR, HER2 and Ki-67 markers. Four subtypes are defined: a) luminal-A - ER positive, PgR positive, HER2 negative and low Ki-67; b) luminal-B - ER positive, PgR negative or low, HER2 negative and high Ki-67; or ER positive, any PgR, HER2 positive and any Ki-67; c) HER2 overexpression (HER2+) - ER negative, PgR negative and HER2 positive; d) Triple negative (TNBC) - ER negative, PgR negative and HER2 negative (Silva et al., 2020).

RNA isolation and RT-qPCR: Tumor fragments were homogenized using an RNA lysis buffer, and RNA was purified using the RNAeasy® Kit (*Qiagen*) with DNAse digestion. The RNA integrity was evaluated on a 1% agarose gel, and RNA concentrations were measured in NanoDrop™ 2000 spectrophotometer (*Thermo Scientific*). Retro-transcription (1,200 ng of RNA) was performed with High Capacity cDNA Reverse Transcription® Kit (*Applied Biosystems*). Optimal primer pairs spanning splice junctions were chosen using the PRIMER-3 and *Oligo Analyzer* software programs (Table 2). The specificity was verified *in silico* and by analyzing PCR products (single-band amplicons). Actin (*ACTB*) and β-2-microglobulin (*B2M*) were used as internal references (housekeeping genes). They were chosen using the TaqMan® Human Endogenous Control Array microfluidic card (*Applied Biosystems*).

Relative gene expression was determined by Real-Time Quantitative Reverse Transcription PCR (RT-qPCR) on a Mastercycler ep RealPlex system (*Eppendorf*). Standard curves were included for efficiency reaction analyses, necessary for the comparative method ($2^{-\Delta\Delta Ct}$). Briefly, 2 µl of cDNA template (15 ng), 2 ρmol of forward and reverse primers, 5 µl of SYBR Green PCR Master Mix (*Applied Biosystems*) were added in a total volume reaction of 10 µl. The sets of primers are shown in Table 2. The

Brazilian Journal of health Review

following PCR program was performed: two minutes at 50 °C, ten minutes at 95 °C (initial denaturation), 15 seconds at 95 °C and one minute at the annealing temperature. These steps were repeated a total of 40 times. The PCR reactions were evaluated using a melting curve analysis according to the manufacturer's instructions.

Statistical analyses: Normalization was conducted using the relative expression of the housekeeping genes. The normalized values were evaluated using the Shapiro-Wilk normality test, which exhibited a non-normal distribution. A non-parametric Mann-Whitney test was chosen to analyze the differences in gene expression between the groups (tumor vs. NC samples, positive lymph nodes vs. negative lymph nodes, histological grade I/II vs. III, ER positive vs. ER negative, PgR positive vs. PgR negative and HER2 positive vs. HER2 negative), and Pearson's R correlation coefficient test was used to associate gene expression with age at diagnosis and tumor size. All statistical analyses were performed using Prism 5 version 5.04 (*GraphPad Software Inc*.). A two tailed *p*-value of <0.05 was considered as statistically significant.

3 RESULTS

To perform gene expression analyses, breast tumors were classified based on histological subtype of invasive ductal carcinoma (IDC, n= 36), and based on the IHC subtype classification (n= 42). The relative expression of *FA-BRCA* genes in IDC and in NC breast tissues are shown in Table 3. *FANCD2* expression was 1.96 higher in IDC than in NC tissues (p= 0.02).

Not significant differences were found between gene expression (*FANCA, C, D2, F, PALB2*) and the clinico-pathological parameters, such as lymph node invasion, tumor grade (I/II vs. III), hormonal receptor status (ER/PgR) and HER2 amplification (positive or negative). *BRCA1* expression was 3.17 higher in PgR positive than in PgR negative tumors (p= 0.03). No correlations were found between gene expression and age at diagnosis and tumor size.

When the samples were classified based on the IHC subtypes, only the analysis between the Luminal-A and Luminal-B groups could be performed due to the limited number of samples in HER2+ and TNBC groups. *BRCA1* expression was 2.66 higher in Luminal-A than in Luminal-B tumors (p= 0.01) (Table 4). *BRCA1* expression was even lower in the TNBC (relative expression= 2.6) and HER2+ (relative expression= 1.7) groups, although statistical analysis was not possible. There is a slight negative correlation between

Luminal-A and TNBC groups in relation to *BRCA1* expression (r= -0,99; p= 0,049). The differences in expression among the other genes were not statistically significant (Table 4).

4 DISCUSSION

Our data indicate that *FANCD2* expression is nearly two times higher in IDC than in NC samples. Once FANCD2 protein is monoubiquitylated by FA core-complex, it translocates to chromatin and co-localizes with BRCA1, BRCA2 and RAD51 in DNA damage sites (Rudland et al., 2010). Ozawa et al. (2010) observed that *FANCD2* expression was significantly higher in colorectal tumors than in normal samples of five patients. In addition, when 30 resected colon cancer specimens were analyzed, 70% presented higher FANCD2 protein expression than in the corresponding normal tissue. Higher *FANCD2* expression in patients (n= 133) with lymph node and liver metastases or recurrence was also observed. Kao et al. (2011) observed that the FA genes expression were significantly elevated in malignant melanomas (n= 40) when compared to normal skin. The melanoma-specific signature was provided by the assembled expression of FANCL and FANCD2, which was at least 50% higher than in normal skin samples. These results indicated that activation of the *FA-BRCA* pathway may contribute to carcinogenesis and resistance to chemotherapy, suggesting a potential therapeutic strategy for melanoma. Ganzinelli et al. (2011) observed that the *FA-BRCA* genes (*BRCA1, FANCA, FANCC, FANCD2* and *FANCF)* expression in ovarian cancer was significantly higher in carcinoma stage I (n= 77) than in borderline stage I (n= 13), suggesting that cancer development is associated with upregulation of genes involved in DNA repair and in the maintenance of genomic stability. Our results agree with Ozawa et al. (2010), Kao et al. (2010) and Ganzinelli et al. (2011), suggesting a possible mechanism of *FA-BRCA* genes also in breast cancer.

However, some studies indicated that a failure to express *FANCD2* may have a role in the process of sporadic breast cancer development (Mathew, 2006; Pejovic et al., 2006; Zhang et al., 2010). These studies suggested that loss of *FANCD2* expression causes chromosome instability and inhibition of normal cell differentiation, playing an important role in malignant transformation and tumorigenesis. Duan et al. (2013) suggested a method based on western blotting to evaluate FANCD2 protein monoubiquitination in tumors. The ratio of FANCD2 foci-negative breast tumors was 29.4%. They hypothesized that if FA-defective tumors are sensitive to ICL agents and PARP inhibitors, this simple test may have widespread applications.

There were no correlations between *FANCD2* expression and the clinico-pathological parameters. Also, Zhang et al. (2010) did not find any association between *FANCD2* expression and patient characteristics and tumor markers (age, ER, PgR, tumor size and lymph node metastasis) in 162 breast cancer patients.

Some studies have reported that *FA-BRCA* pathway activity is closely correlated with the levels of FA gene transcripts (Taniguchi et al., 2002; Hoskins et al., 2008). In addition, FANCD2-FANCI heterodimer association is a well-established hallmark for pathway activation (Kee and D'Andrea, 2010). These reports suggest that the *FA-BRCA* pathway may activate oncogenesis, in addition to its role as TSG (Condie et al., 2002; Nitta et al., 2010). This pathway activation provides resistance to increased endogenous DNA damage, commonly observed in oncogenic states (Chen et al., 2007), and confers survival advantages to tumor cells. In agreement with this hypothesis, activation of the *FA-BRCA* pathway was associated to resistance to DNA-damaging agents, such as cyclophosphamide (Yarde et al., 2009). One possible explanation for the observed *FANCD2* upregulation in the present study comes from a melanoma pathogenesis analysis, in which the E2F transcription factor and NF-kB seemed to activate *FANCD2* by binding to promoter or consensus promoter response elements (Hoskins et al., 2008; Yarde et al., 2009).

We observed *BRCA1* gene expression 3.17 times higher in PgR positive than in PgR negative breast tumors. This result is in accordance with Verma et al. (2018), who observed a positive significant association between BRCA1 nuclear protein (IHC) expression and PgR status ($p= 0.001$). However, Zhang et al. (2012) found no correlation among BRCA1 protein expression (IHC) and ER, PgR, HER2, WWOX (WW domain containing oxidoreductase) expression, Ki-67 proliferation rate and the histological grade ($P> 0.05$). Also, Kumar et al. (2017) described no statistical association among BRCA1 reduced/moderate to strong protein expression and PgR expression ($p< 0.083$) in a study with 114 breast cancer patients.

In our study, *BRCA1* gene presented the highest level of expression from all evaluated genes in both tumor and NC samples (Table 3), although the differences in the expression between these two groups were not statistically significant. Additionally, we observed a 2.7-fold loss of expression of *BRCA1* in the Luminal-B (relative expression= 2.89) group compared to the Luminal-A (relative expression= 7.70) group (Table 4). BRCA1 expression was even lower in the TNBC (relative expression= 2.6) and HER2+ (relative expression= 1.7) groups, with a slight negative correlation between Luminal-A and

TNBC groups (r= -0,99; p= 0,049) (Fig. 1). These results are in accordance with Ganzinelli et al. (2011), which revealed a down-regulation of *BRCA1* in more aggressive stages of ovarian tumors. Also, in a previous study (Ribeiro et al., 2013), we observed a lower expression of *BRCA1* in a group of 80 TNBC in comparison to 70 Luminal-A tumors. Taken together, these results suggest that *BRCA1* downregulation occurs in the most aggressive subtypes of the disease, which may be responsible for increased genomic instability. We did not observe any difference in the expression of the other FA genes between the luminal groups.

BRCA1 mRNA expression can be exploited for the treatment of breast tumors with different classes of drugs. Just as in FA cells, Van der Groep et al. (2008) suggested the hypersensitivity of cells lacking BRCA1/2 proteins to DNA cross-linking agents, such as cisplatin. *BRCA1* mutations or *BRCA1* reduced expression may maintain sensitivity to platinum-based chemotherapy. Thus, *BRCA1* may favorably influence prognosis (Lambie et al., 2003). Alan and D'Andrea (2010) delineated that tumors with *BRCA1*/2 mutations depend to a large degree on the Base Excision Repair (BER), and the enzyme Poly-ADP ribose polymerase 1 (PARP1) is critical for BER process. Thus, tumors defectives in homologous recombination genes (such as *FA-BRCA* genes) are also hypersensitive to PARP inhibitors (Bryant and Helleday, 2006; Fong et al., 2009), expanding the utility of this class of drugs to non *BRCA1*-mutated patients (Edwards et al., 2008). Gao et al. (2013) observed that *BRCA1* upregulation is associated with DNA repair-mediated resistance to cisplatin, and that *BRCA1* downregulation confers resistance to taxanes, such as docetaxel and paclitaxel. They have also clinically confirmed that *BRCA1* mutations or expression alterations affect the response to chemotherapy based on cisplatin or paclitaxel and have a survival influence in a number of different types of cancer, including breast cancer. Therefore, *BRCA1* downregulation in Luminal-B breast cancer patients may be a valid indicator of the success of cisplatin-based treatment. In addition, docetaxel-based therapy should be avoided, and Luminal-B patients may benefit from treatment with PARP inhibitors. Yuanming et al. (2013) found that the decreased expression of the ERCC1 (excision repair cross-complementation group 1) and BRCA1 proteins is associated with poor prognosis in metastatic colorectal cancer and that these proteins could be used as prognostic biomarkers.

Among the 36 IDC patients, 19 (53%) received some type of cyclophosphamide-based adjuvant regimen (which may confer resistance in these patients), 13 (36%) received

a hormonal therapy (tamoxifen or aromatase inhibitors) alone, and 4 (11%) did not received any adjuvant therapy. In addition, four Luminal-B patients received adjuvant paclitaxel-based treatments, which may result in the development of resistance. It is of importance to analyze the expression of DNA repair genes in breast cancer patients, such as the *FA-BRCA* pathway, because these genes may interfere with the response to treatment and, consequently, improve the disease-free survival (DFS) and overall survival (OS).

There is a need for new predictive biomarkers in breast cancer, especially for more aggressive tumors (such as Luminal-B, HER2+ and TNBC), with the aim of better distinguishing which patients will benefit from standard chemotherapy regimens. The *FA-BRCA* pathway is an important source of new predictive biomarkers for breast cancer.

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Compliance with Ethical Standards

Ethical approval

This research has been approved by Ethical Committee of Federal University of Paraná. All procedures performed in the study involving human participants, were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Author contributions

SFVOM: collected clinical information from the medical records; performed molecular tests and statistical analysis; article writing and submission.

LS: performed molecular tests; assisted during article writing and submission.

LGS: performed molecular tests and statistical analysis; assisted during article writing and submission.

MEM: performed molecular tests; assisted during article writing and submission.

CAU: selected patients and collected biological samples; obtained informed consent from patients.

RSL: selected patients and collected biological samples; obtained informed consent from patients.

IJC: assisted during article writing and submission.

EMSFR: assisted during article writing and submission; coordinated the research group.

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Brazilian Journal of health Review

Table 1 – Clinico-pathological information.

Parameter	Number of Patients	%
Age	46 (mean 59,3 years)	100
Histological subtypes		
IDC	36	78.26
ILC	3	6.52
Mixed (IDC + ILC)	4	8.70
Others[a]	3	6.52
IHC subtypes		
Luminal-A	23	54.80
Luminal-B	14	33.30
HER2 +	2	4.80
TNBC	3	7.14
Not-informed	4	-
Lymph node metastasis		
Present	21	48.84
Absent	22	51.16
Not-informed	3	-
Tumor grade		
I	6	13.64
II	28	63.64
III	10	22.72
Not-informed	2	-
Tumor size		
≤ 20 mm	20	45.45
> 20 mm	24	54.55
Not-informed	02	-
Estrogen receptor (ER)		
Positive	37	84.09
Negative	7	15.91
Not-informed	2	-
Progesterone receptor (PgR)		

Brazilian Journal of health Review

Parameter	Number of Patients	%
Positive	38	86.36
Negative	6	13.64
Not-informed	2	-
HER2 amplification		
Positive	9	21.43
Negative	33	78.57
Not-informed	4	-

Legend: IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; IHC, immunohistochemical; TNBC, triple negative breast cancer; mm, millimeters; [a], mucinous carcinoma, tubular-lobular carcinoma, pleomorphic carcinoma.

Table 2 – Characteristics of primers used in RT-qPCR.

Gene	Sense	Sequence	Amplicon Length (bp)
ACTB	F	5'-GATGCAGAAGGAGATCACTGC	75
	R	5'-AGTACTTGCGCTCAGGAGGA	
B2M	F	5'-TGACTTTGTCACAGCCCAAGATA	77
	R	5'-CGGCATCTTCAAACCTCCA	
BRCA1	F	5'- GCCAGAAAACACCACATCAC	99
	R	5'- CAGTGTCCGTTCACACACAA	
FANCA	F	5'- GAGGTTCTTCAGTCATACCCTGA	84
	R	5'- TCTCTCTGCATCTGAACAGCA	
FANCC	F	5'- CCAGCCAGAGTTCTTTGAGG	90
	R	5'- CGAAGCCAGAGGCAGACTAC	
FANCD2	F	5'- CCCATCTGCTATGATGATGAA	81
	R	5'- CGTATTTGCTGAGGGGATATG	
FANCF	F	5'- GCTAGTCCACTGGCTTCTGG	95
	R	5'- GGTGGCGGCTAGTCACTAAA	
PALB2	F	5'- TGGGACCCTTTCTGATCAAC	83
	R	5'- GGGGCATCAAAAATTGGTTT	

Legend: F, forward; R, reverse; bp, base pairs.

Brazilian Journal of health Review

Table 3 – Relative expression of *FA-BRCA* genes in the IDC and NC breast tissue samples

	FANCA	***FANCC***	***FANCD2***	***FANCF***	***BRCA1***	***PALB2***
IDC	3.12 (34)	1.50 (34)	1.23 (35)	0.80 (34)	5.15 (34)	0.94 (35)
NC	1.32 (8)	1.36 (10)	0.63 (10)	0.74 (8)	2.30 (10)	0.75 (10)
IDC/NC	2.36	1.10	1.96*	1.08	2.24	1.25

Legend: IDC, invasive ductal carcinomas; NC, non-compromised breast samples; ( ), number of samples; *, statistical significance: $p = 0.02$.

Table 4 – *FA-BRCA* gene expression between luminal immunohistochemical subtypes.

	FANCA	***FANCC***	***FANCD2***	***FANCF***	***BRCA1***	***PALB2***
Luminal-A	5.33 (21)	1.47 (21)	1.77 (22)	1.44 (21)	7.70 (21)	1.01 (22)
Luminal-B	2.98 (14)	1.54 (14)	1.13 (14)	0.69 (14)	2.89 (14)	0.87 (14)
LA/LB	1.79	0.95	1.57	2.09	2.66*	1.16

Legend: ( ), number of samples; LA/LB, Luminal-A/Luminal-B; *, statistical significance: $p = 0.01$.