Molecular analysis of EGFR gene mutation and PD-L1 expression in a Brazilian cervical cancer cohort

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INTRODUCTION

Cervical cancer (CC) represents the third most commonly diagnosed cancer and the fourth cause of cancer death in women worldwide[1,2]. There are two main types of CC: squamous cell carcinoma (SCC), that accounts for 80-90% of the cases and adenocarcinoma (ACA), which represents 10-20% of CC histology. Although preventive strategies are cardinal, early diagnosis and an effective treatment are primordial for the reduction of morbidity and mortality.

EGFR receptors are expressed by CC cells and when autofosforilated activation of cancer signaling pathways is promoted, but data from mutational status of EGFR during cervical carcinogenesis differ in the literature. EGFR receptor could be a target therapy in CC as it is in lung cancer[3,4,5].

Also, the programmed death-1/programmed death-ligand-1 (PD-1/PD-L1) immune regulatory axis has emerged as a promising new target for cancer therapeutics, with lasting responses seen in the treatment of metastatic renal, lung cancer and melanomas. Tumor surface expression of PD-L1 has been found to correlate with objective responses to anti-PD-L1 immunotherapies.

Due to the high mortality of the advanced CC, the identification of a specific population that would benefit from target therapy is of the utmost importance.

AIM

To investigate the EGFR mutations and PD-L1 expression in CC and evaluate their prognostic significance in those patients.

MATERIAL AND METHODS

A cohort of CC from 2011 INCA were evaluated for EGFR mutation and PD-L1 expression. DNA samples were isolated from formalin-fixed, paraffin-embedded samples of CC patients. Direct sequence analysis of the PCR products of EGFR gene was used in order to evaluate activating mutations. PD-L1 expression were evaluated through immunohistochemistry of tissue microarrays (TMA) of the same CC cases. Epidemiologic data were collected. 123 SCC and 61 ACA matched our selection criteria for DNA extraction and TMA preparation.

TABLE 1:Clinical and Epidemiologic variables by cancer type

Clinical and epidemiological variables	Squamous cell carcinoma (N=123)	Adenocarcinoma (N=61)	Total (N=184)	p-value
Age, years	52.33 (±15.3)	53.69 (±14.27)	52.78 (±14.96)	0.546
Age, years				0.783
18-30	26 (21.1%)	11 (18.0%)	37 (20.1%)	
30-50	66 (53.7%)	32 (52.6%)	98 (53.3%)	
>50	31 (25.2%)	18 (29.5%)	49 (26.6%)	
Age				0.467
≤70	104 (84.6%)	54 (88.5%)	158 (85,9%)	
>70	19 (15.4%)	7 (11.5%)	26 (14,1%)	
Menarch, year	13.07 (±1.80)	13.38(±1,79)	13.17 (± 1.80)	0.258
Menarch, years				0.925
≤ 14	92 (76.0%)	46 (76.7%)	138 (76.2%)	
> 15	29 (24.0%)	14 (23.3%)	43 (23.8%)	
Sexual initiation, years				**0.004**
< 15	38 (31.9%)	5 (8.8%)	43 (24.4%)	
15-20	62 (52.1%)	41 (71.9%)	103 (58.5%)	
> 20	19 (16.0%)	11 (19.3%)	30 (17.0)	
Sexual partners				**0.013**
0 ou 1	32 (37.4%)	26 (46.4%)	58 (33.5%)	
≥ 2	85 (72.6%)	30 (53.6%)	115 (66.5%)	
Pregnancy				0.078
Yes	120 (98.4%)	57 (93.4%)	177 (96.7%)	
No	2 (1.6%)	4 (6.6%)	6 (3.3%)	
Menopause				0.143
Yes	60 (49,2%)	37 (60.7%)	86 (47.0%)	
No	62 (50.8%)	24 (39.3%)	37 (53.0%)	
Time between last cervical cytology and cervical cancer, year	2.89 (±3.82)	2.96 (±4.02)	2.92 (±3.88)	0.923
Time between last cervical cytology and cervical cancer, year				0.725
≤1	11 (15.7%)	5 (10.9%)	16 (13.8%)	
1-3	43 (61.4%)	31 (67.4%)	74 (63.8%)	
>3	16 (22.9%)	10 (21.7%)	26 (22.4%)	
Smoking				0.063
Yes	58 (47.2%)	20 (32.8%)	78 (42.4%)	
No	65 (52.8%)	41 (67.2%)	106 (57.6%)	
Comorbidies				0.318
Yes	47 (38.2%)	28 (45.9%)	75 (40.8%)	
No	76 (61.8%)	33 (54.1%)	109 (59.2%)	
Contraceptive use				0.231
Yes	73 (60.3%)	41 (69.5%)	114 (63.3%)	
No	48 (39.7%)	18 (30.5%)	66 (36.7%)	
FIGO classification				0.073
Ia	1 (0.8%)	0 (0.0%)	1 (0.5%)	
Ib1	13 (10.6%)	15 (24.6%)	28 (15.2%)	
Ib2	9 (7.3%)	9 (14.8%)	18 (9.8%)	
IIa	5 (4.1%)	1 (1.6%)	6 (3.3%)	
IIb	35 (28.5%)	20 (32.8%)	55 (29.9%)	
IIIa	1 (0.8%)	0 (0.0%)	1 (0.5%)	
IIIb	50 (40.7%)	13 (21.3%)	63 (34.2%)	
IVa	6 (4.9%)	2 (3.3%)	8 (4.3%)	
IVb	3 (2.4%)	1 (1.6%)	4 (2.2%)	
FIGO classification				**0.021**
Ia e Ib1	14 (11.4%)	15 (24.6%)	29 (15.8%)	
Ib2-IVb	109 (88.6%)	46 (75.4%)	15 (84.2%)	
Visible tumor size, cm				**0.003**
≤ 4	39 (33.1%)	33 (55.9%)	72 (40.7%)	
> 4	79 (66.9%)	26 (44.1%)	105 (59.3%)	
Positive lymph nodes in surgical patients				0.782
Yes	4 (18.2%)	3 (15.0%)	7 (16.7%)	
No	18 (81.8%)	17 (85.0%)	35 (83.3%)	
Surgery				**0.031**
Yes	23 (18.75)	20 (32.8%)	43 (23.4%)	
No	100 (81.3%)	41 (67.2%)	141 (76.6%)	
Chemotherapy				0.234
Yes	77 (64.2%)	33 (55.0%)	110 (61.1%)	
No	43 (35.8%)	27 (45.0%)	70 (38.8%)	
Radiotherapy				0.437
Yes	95 (78.5%)	44 (73.3%)	139 (76.8%)	
No	26 (21.5%)	16 (26.7%)	42 (23.2%)	
Brachytherapy				0.590
Yes	71 (59.2%)	38 (63.3%)	109 (60.6%)	
No	49 (40.8%)	22 (36.7%)	71 (39.4%)	
Radiochemotherapy alone				0.310
Yes	97 (81.5%)	45 (75.0%)	142 (79.3%)	
No	22 (18.5%)	15 (25.0%)	37 (20.7%)	
Progression or recurrence				0.959
Yes	63 (51.2%)	31 (50.8%)	94 (51.1%)	
No	60 (48.8%)	30 (49.2)	90 (48.9%)	
Death				0.228
Yes	65 (53.7%)	27 (44.3%)	92 (50.5%)	
No	56 (46.3%)	34 (55.7%)	90 (49.5%)	
Last visit status				0.301
With cancer	4 (7.0%)	4 (11.8%)	8 (8.8%)	
Without cancer	51 (89.5%)	30 (88.2%)	81 (89.0%)	
No information	2 (3.5%)	0 (0.0%)	2 (2.2%)	

Continuous variables are presented as mean ± standard deviation; categorical variables as frequency (percentage).
A p-value in bold font indicates a significant difference between groups

RESULTS

184 CC patients stage IA to IV with age between 18-80 years from 2011 were included. Table 1 demonstrates the clinical and epidemiological variables by cancer type. SCC and ACA are similar with the exception of age at sexual initiation, number of sexual partner, FIGO classification, tumor size and surgical treatment. Table 2 demonstrates pathological characteristics by cancer type, SCC and ACA are not similar in many variables: cancer and stromal percentage, necrosis, mitosis, inflammation type and site, pleomorphism and PD-L1 expression. Regarding PD-L1 expression, within ACA (61 cases), 8 had insufficient material, 58.5% were negative, 10 (18.9%) presented minimal PD-L1 expression of <5%, 4 (7.5%) presented PD-L1expression between 5-25%, 3 (5.7%) between 25-50%, 2 (3.8%) between 50-75% and 3 (5.7%) between 75-100% of positivity. Three cases had heterogeneity expression. Within SCC, (123 cases), 20 had insufficient material, 42/103 (40.8%) were negative, 11 (10.7%) presented minimal PD-L1 expression of <5%, 19 (18.4%) presented PD-L1expression between 5-25%, 18 (17.5%) between 25-50%, 6 (5.8%) between 50-75% and 7 (6.8%) between 75-100% of positivity. Heterogeneity of PD-L1 expression was seen in 11 cases. Overall, 41.5% of ACA and 59.2% of SCC had positive PD-L1 expression. EGFR exon 18, 19, 20 and 21 were investigated and mutation was found in 1 (0.9%) of 112 SCC. None ACA presented EGFR mutation. The mutation occurred at exon 18 by a replacement of the amino acid Glutamate with Glutamine at position 711 (E711Q ou c.2131G>C).

TABLE 2: Pathologic variables by cancer type

Pathological review	Squamous Cell cancinoma (N=123)	Adenocarcinoma (N=61)	Total (N=184)	p-value
Cancer percentage	77,30 (±11.85)	81.17 (±10,18)	78,57 (±11.45)	**0,024**
Stromal percentage	16.80 (±10.37)	11.17 (±10.37)	14,71 (±9.77)	**<0,001**
Mitosis	24.45 (±48.99)	12.76 (±15.38)	20,79 (±41.82)	**0,020**
Stromal type				**0.008**
Fibrous	54 (45.0%)	14 (23.3%)	68 (37.8%)	
Muscular	66 (55.0%)	45 (75.0%)	111 (61.7%)	
Necrosis				0.197
Withou necrosis	21 (17.5%)	13 (21.7%)	34 (18.9%)	
Mild	70 (58.3%)	25 (41.7%)	95 (52.8%)	
Moderate	20 (16.7%)	15 (25.0%)	35 (19.4%)	
High	9 (7.5%)	7 (11.7%)	16 (8.9%)	
Quantity of inflammation				0.635
Mild	55 (45.8%)	32 (53.3%)	87 (48.3%)	
Moderate	47 (39.2%)	18 (30.0%)	65 (36.1%)	
High	15 (12.5%)	9 (15.0%)	24 (13.3%)	
Inflammation type				**0.004**
Lymphocyte	30 (25.0%)	6 (10.0%)	36 (20.0%)	
Polymorph	6 (5.0%)	5 (8.3%)	11 (6.1%)	
Both	17 (14.2%)	20 (33.3%)	37 (20.6%)	
Inflammation site				**0.005**
Intratumoral	81 (71.1%)	34 (56.7%)	115 (66.1%)	
Peritumoral	14 (12.3%)	9 (15.0%)	23 (13.2%)	
Stromal	11 (9.6%)	16 (26.7%)	27 (15.5%)	
Intratumoral and Stromal	8 (7.0%)	0 (0.0%)	8 (4.6%)	
Pleomorphism				**<0.001**
Mild	2 (1.7%)	9 (15.0%)	11 (6.1%)	
Moderate	72 (60.0%)	40 (66.7%)	112 (62.2%)	
High	46 (38.3%)	11 (18.3%)	57 (31.7%)	
Differentiation				0.054
Mild	11 (9.6%)	13 (21.7%)	24 (13.7%)	
Moderate	77 (67.0%)	31 (51.7%)	108 (61.7%)	
Hight	27 (23.5%)	15 (25.0%)	42 (24.0%)	
PD-L1 expression				**0.040**
Without expression	42 (40.8%)	31 (58.5%)	73 (46.8%)	
Up to 5%	11 (10.7%)	10 (18.9%)	21 (13.5%)	
5-25%	19 (18.4%)	4 (7.5%)	23 (14.7%)	
25-50%	18 (17.5%)	3 (5.7%)	21 (13.5%)	
50-75%	6 (5.8%)	2 (3.8%)	8 (5.1%)	
75-100%	7 (6.8%)	3 (5.7%)	10 (6.4%)	
PD-L1 expression heterogeneity				0.251
Yes	11 (8.9%)	3 (4.9%)	14 (7.6%)	
No	92 (74.8%)	52 (85.2%)	144 (78.3%)	
PD-L1 expression intensity				0.064
Without expression.	42 (40.8%)	31 (58.5%)	73 (46.8%)	
Weak	38 (36.9%)	16 (30.2%)	54 (34.6%)	
Moderate	21 (20.4%)	4 (7.5%)	25 (16.0%)	
High	2 (1.9%)	2 (3.8%)	4 (2.6%)	

Continuous variables are presented as mean ± standard deviation; categorical variables as frequency (percentage).
A p-value in bold font indicates a significant difference between group
PD-L1, programmed death-ligand.

CONCLUSION

The presented results indicate that EGFR mutations is uncommon in CC and it is unlikely to be useful for cancer treatment. PD-L1 expression could be considered as a potential biomarker for CC and a potential target for immunotherapy.

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