MITOCHONDRIAL ENZYMES CORRELATION WITH BREAST CANCER CLINICOPATHOLOGICAL PARAMETERS/ A STUDY IN SULAIMANI CITY-IRAQ

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Submitted: 30/9/2017; Accepted: 7/2/2018; Published 15/4/2018

ABSTRACT

Background

Thousands of researches are done for breast cancer, but still cancer epithelial cells relationship with the surrounding stromal cells is a great topic for researchers which is termed the tumor microenvironment.

Objectives

This study is designed to confirm that presence of the two main mitochondrial enzymes that act in ketone bodies production and utilization and their correlation with the clinicopathological parameters and tumor aggressiveness.

Materials and Methods

This cross sectional study was carried out in Iraq in which 40 selected cases of breast invasive ductal carcinoma not otherwise specified were stained immunohistochemically for two mitochondrial enzymes; HMGCS2 and ACAT1involved in ketone bodies production and utilization respectively and correlate their positivity with clinicopathological parameters of the breast cancer as patient's age, estrogen and progesterone receptors, HER2, molecular types, tumor grade and lymph node metastasis.

Results

Out of 40 cases, 50% are in the age range of 41-50 years. Grade I seen in 7%, grade II in 23% and grade III in 70% of cases. Regarding lymph node status, 10% are N2 while 28% are N3. ACAT1 shows stromal significant positivity with N3 only (P. = 0.01). HMGCS2 shows a highly significant epithelial positivity with N2 (P. = 0.004) and a significant stromal positivity with low grade tumors (P. = 0.04).

Conclusion

No significant correlation between ketone bodies production or utilization with the studied clinicopathological parameters apart from tumor grade and lymph node status, indicating that aggressive and metastatic tumors are more capable of self dependence for feeding themselves with ketone bodies.

Keywords: *Ketone bodies, Breast cancer, Mitochondrial enzymes, ACAT1, HMGCS2* .

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https://doi.org/10.17656/jsmc.10149

INTRODUCTION

Carcinomas are cancers formed from epithelial tissue. This tissue is not vascularized, which prevents the tumor from growing beyond 2 mm in diameter but cancers are highly complex tissues composed of cancer epithelial cells and its surrounding stroma, which is built up by various types of mesenchymal cells and an extracellular matrix [(1)]. Cancers change their metabolism and rely on glycolysis even if oxygen is available and this phenomenon is termed aerobic glycolysis. This conjecture increases the cancer cells reliance on glucose, potentially reducing the cancer cells ability to utilize non-glucose substrates. The main alternatives to glucose are high-energy breakdown products of hepatic lipid metabolism, collectively termed ketone bodies. The net result is liberation of high level of lactic acid and ketone bodies which then used as fuel by cancer cells for oxidative phosphorylation [(2)].

This study is designed to highlight immunohistochemically two mitochondrial enzymes in both compartments; breast cancer epithelial cells and cancer associated fibroblasts. The first enzyme is HMGCS2; an enzyme works initially in ketone bodies production by adding acetyl-CoA molecule to acetoacetyl-CoA to form HMG-CoA which in subsequent steps changes into β-δ hydroxybutarate [(3)]. The other enzyme is ACAT1 also known as thiolase which is the last enzyme involved in ketone bodies utilization changing acetoacetyl-CoA into two molecules of acetyl-CoA that then enter the citric acid cycle [(4)].

MATERIALS AND METHODS

This retrospective cross sectional study was carried out in the period from March 2015 to July 2016, whereby paraffin-embedded tissue blocks of forty female patients with invasive ductal carcinoma (NOS) of the breast were collected and reviewed from different hospitals in Sulaimani city in Iraq. Previous histopathology reports were collected. Data including age of the patient, tumour size, lymph node status, tumor grade, oestrogen receptor (ER) positivity, progesterone receptor (PR) positivity, HER2, Ki-67 and other pathological parameters were all recorded. Molecular types of breast cancer were identified using the five categorization system: luminal A, luminal B/HER2 negative, luminal B/HER2 positive, triple negative and HER2 over-expressing.

Two other immunohistochemical markers; primary polyclonal antibody ACAT1 (SC-161307) and primary monoclonal antibody HMGCS2 (SC-367092) were used with a dilution at 1:100 using the ImmunoCruz™ ABC Staining Systems at Ph=6.1. The intensities were scored in cancer epithelial cells and cancer associated fibroblasts stromal cells as follow: no stain, low, intermediate and high. The immunohistochemical results were correlated with all the clincopathological parameters of the each case.

Data collected and analysed using SPSS version, Chi-square test, and Z test for proportion. Level of significant < 0.05 is used.

RESULTS

A total of 40 cases of invasive ductal carcinoma (NOS) were studied. The age of the patients ranged from 31 to 88 years, (mean ± S.D. = 47.83 ± 12.04), 50% of cases were in the age range of 41-50 years, 22.5 % were ≤ 40 years (Figure 1).

The present shows grade I in 3 cases (7%), grade II in 9 cases (23%), and grade III in 28 cases (70%), (Figures 2 and 3).

Eleven cases (28%) were N0, 14 cases (34%) were N1, 4 cases (10%) were N2, and 11 cases (28%) were N3, (Figure 4).

In this study molecular typing was done based on the collected reports data; 14 cases (34%) were luminal A, 9 cases (23%) were luminal B/HER2negative, 8 cases (20%) were luminal B/HER2positive, 5 cases (13%) were triple negative and 4 cases (10%) were HER2 over expressing, (Figure 5).

ACAT1 positivity was scored in both the cancer epithelial cells and stromal cancer associated fibroblasts, (Figure 6).

HMGCS2 was scored in cancer epithelial cells and stromal cancer associated fibroblasts, (Figures 7).

Statistical analysis of epithelial and stromal intensity of both ACAT1 and HMGCS2 with all collected clinicopathological parameters was done. No significant correlation with age groups, lymphocytic infiltration, in-situ component and lymphovascular infiltration was found. Table 1 shows no corrleation with ACAT1.

The same antibodies were also correlated with ER, PR, HER2, Ki-67, molecular subtypes, tumor size, lymph node status and tumor grade, statistically no significant correlation was found between them, apart from lymph node status with ACAT1 stromal intensity in cancer associated fibroblasts and with HMGCS2 epithelial intensity, also tumor grade correlates with HMGCS2 stromal intensity, (Tables 2, 3 and 4).

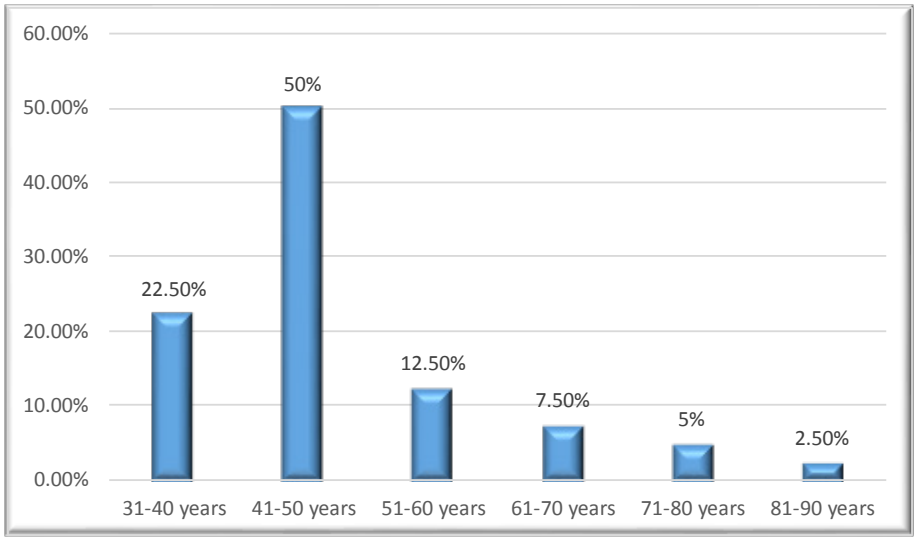

Figure 1. Age distribution in 40 patients.

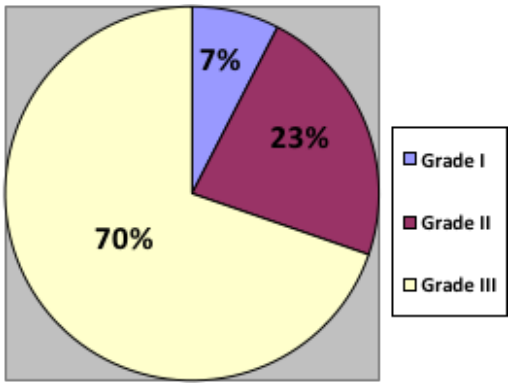

Figure 2. Tumor grade.

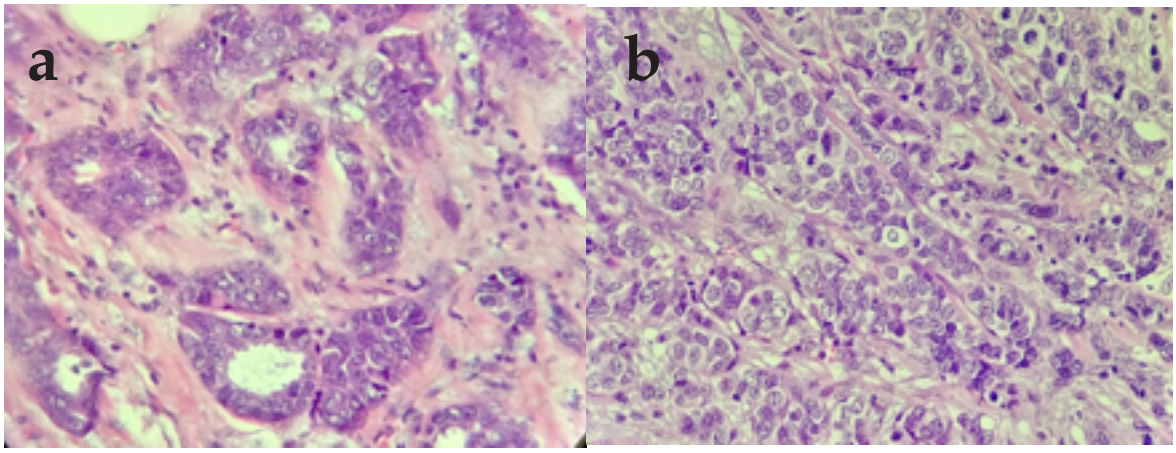

Figure 3. Invasive ductal carcinoma, (a) Grade I, (b) Grade III.

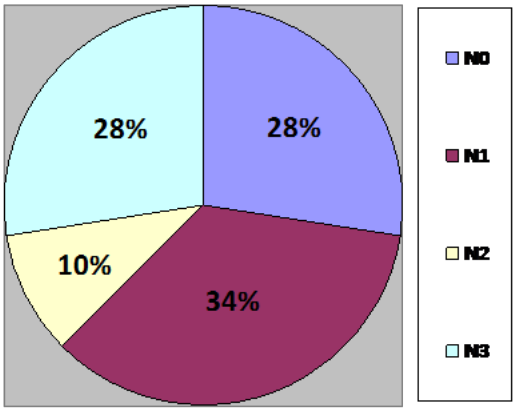

Figure 4. Lymph node status.

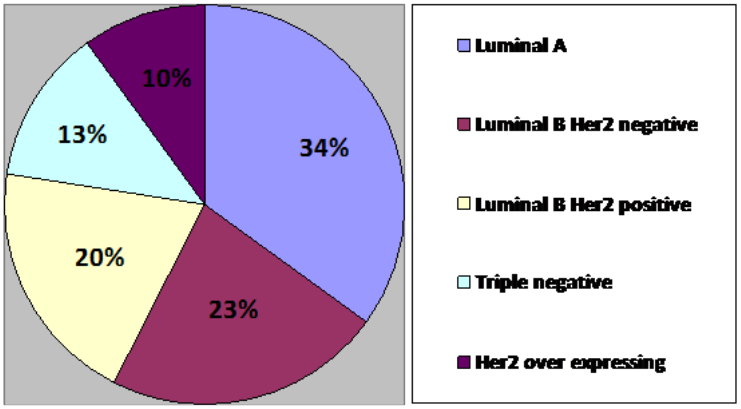

Figure 5. Molecular subtypes.

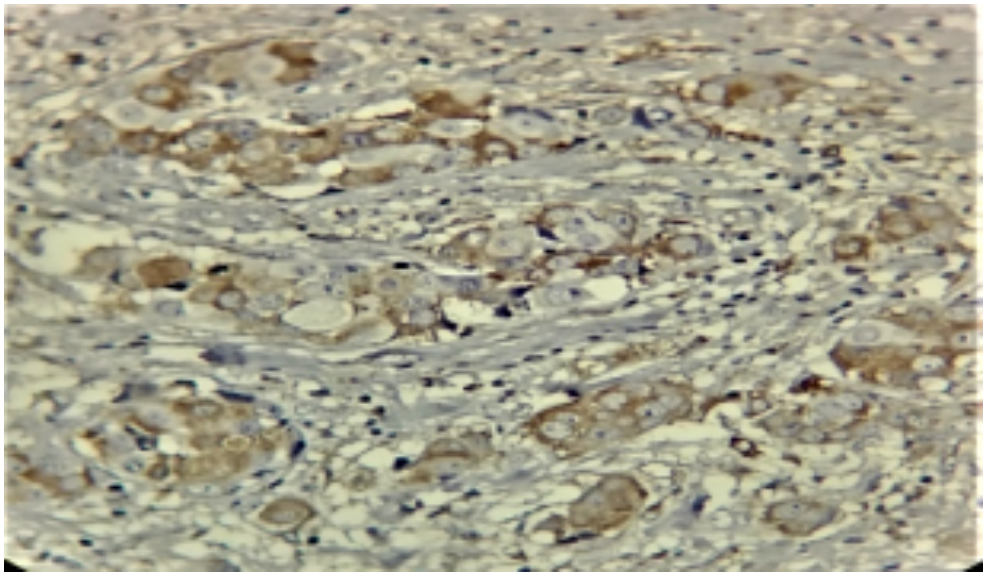

Figure 6. Staining intensity is high in both cancer epithelial cells and cancer associated fibroblasts in high grade breast cancer (ACAT1, X400)

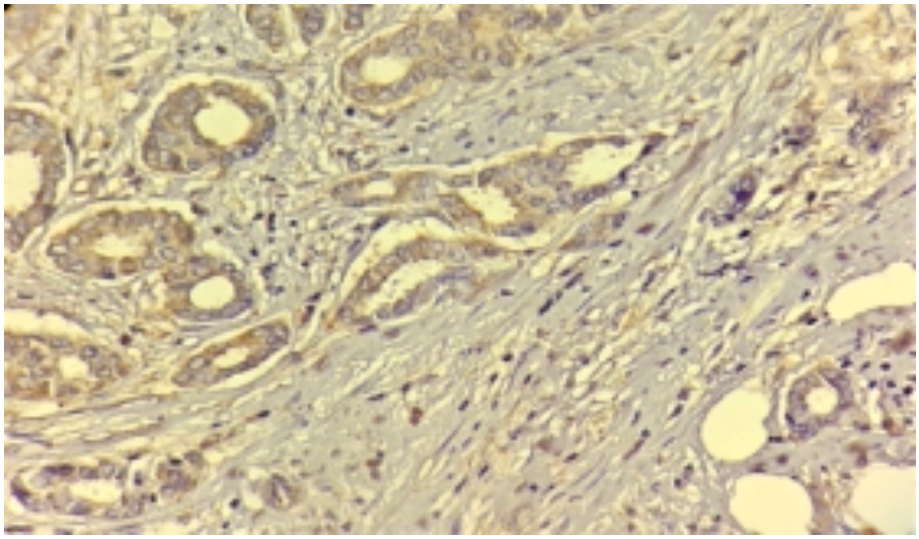

Figure 7. Intermediate staining intensity of both cancer epithelial cells and cancer associated fibroblast in grade II breast cancer. (HMGCS2, X400).

Table 1. Correlation of ACAT1 stromal intensity with clinicopathological parameters.

Parameters		ACAT1 stromal intensity			P - value
		Low	Intermediate	High	
		No.	No.	No.	
Age	**≤ forty**	3	3	2	0.54
	>forty	8	8	14	
Lymphocytic infiltration	**absent**	0	0	1	0.82
	mild	6	4	8	
	moderate	4	4	4	
	prominent	1	2	1	
	Not mentioned	0	1	2	
In situ	**absent**	5	1	8	0.16
	present	6	8	7	
	Not mentioned	0	2	1	
Lymphovascular invasion	absent	4	5	5	0.49
	present	7	5	11	
	Not mentioned	0	1	0	

Table 2. ACAT1 stromal intensity correlation with opathological parameters.

Parameters		ACAT1 stromal intensity						P- value
		low		intermediate		high		
		No.	%	No.	%	No.	%	
ER	Negative	3	30.0	2	20.0	5	50.0	0.72
	Positive	10	33.3	9	30.0	11	36.7	0.72
PR	Negative	4	25.0	5	31.3	7	43.8	0.70
	Positive	9	37.5	6	25	9	37.5	0.70
HER2	Negative	8	28.6	8	28.6	12	42.9	0.71
	Positive	5	41.7	3	25.0	4	33.3	0.71
	can not be assessed	0	0.0	1	50.0	1	50.0	0.56
Ki-67	low grade	1	100	0	0.0	0	0.0	0.35
	intermediate	7	46.7	4	26.7	4	26.7	0.27
	high grade	5	22.7	6	27.3	11	50.0	0.26
Molecular subtypes	luminal A	7	50.0	3	21.4	4	28.6	0.22
	luminal B HER2 negative	0	0.0	4	44.4	5	55.6	0.06
	luminal B HER2positive	4	50.0	2	25.0	2	25.0	0.46
	triple negative	1	20.0	1	20.0	3	60.0	0.61
	HER2 over-expressing	1	25.0	1	25.0	2	50.0	0.90
T	T1	3	45.5	1	18.2	3	36.4	0.65
	T2	10	40.0	6	24.0	9	36.0	0.42
	T3	0	0.0	4	50.0	4	50.0	0.06
	T4	0	0.0	0	0.0	0	0.0	
N	N0	5	45.5	2	18.2	4	36.4	0.51
	N1	6	42.9	2	14.3	6	42.9	0.34
	N2	2	50.0	1	25.0	1	25.0	0.71
	N3	0	0.0	6	54.5	5	45.5	0.01
Grade	low grade	2	66.7	0	0.0	1	33.3	0.35
	intermediate	3	33.3	3	33.3	3	33.3	0.87
	high grade	8	28.6	8	28.6	12	42.9	0.71

Table 3. HMGCS2 epithelial intensity with pathological parameters.

Parameters		HMGCS2 epithelial intensity						P- value
		low		intermediate		high		
		No.	%	No.	%	No.	%	
Molecular subtypes	luminal A	1	7.1	6	42.9	7	55.6	0.34
	luminal B HER2 negative	0	0.0	4	44.4	5	55.6	0.83
	luminal B HER2positive	0	0.0	4	50.0	4	50.0	0.74
	Triple negative	0	0.0	2	40.0	3	60.0	0.92
	HER2 over-expressing	0	0.0	0	0.0	4	100.0	0.19
T	T1	0	0.0	2	28.6	5	71.4	0.67
	T2	1	4.0	10	40.0	14	56.0	0.73
	T3	0	0.0	4	50.0	4	50.0	0.74
N	N0	0	0.0	5	45.5	6	54.5	0.77
	N1	0	0.0	5	35.7	9	64.3	0.66
	N2	1	25.0	0	0.0	3	75.0	0.004
	N3	0	0.0	6	54.5	5	45.5	0.45
Grade	low grade	0	0.0	0	0.0	3	100.0	0.30
	intermediate	1	11.1	2	22.2	6	66.7	0.10
	high grade	0	0.0	14	50.0	14	50.0	0.06

Table 4. Correlation between HMGCS2 stromal intensity with pathological parameters.

Parameters		HMGCS2 stromal intensity						P- value
		low		intermediate		high		
		No.	%	No.	%	No.	%	
ER	Negative	0	0.0	2	20.0	8	80.0	0.29
	Positive	2	6.7	12	40.0	16	53.3	0.29
PR	Negative	0	0.0	4	25.0	12	75.0	0.21
	Positive	2	8.3	10	41.7	12	50.0	0.21
HER2	Negative	1	3.6	13	46.4	14	50.0	0.06
	Positive	1	8.3	1	8.3	10	83.3	0.06
	can not be assessed	0	0.0	0	0.0	2	100.0	0.49
Ki-67	low grade	0	0.0	1	100.0	0	0.0	0.38
	intermediate	2	13.3	6	40.0	7	46.7	0.12
	high grade	0	0.0	7	31.8	15	68.2	0.20
Molecular subtypes	luminal A	1	7.1	7	50.0	6	42.9	0.26
	luminal B HER2 negative	0	0.0	4	44.4	5	55.6	0.11
	luminal B HER2positive	1	12.5	1	12.5	6	75.0	0.22
	triple negative	0	0.0	2	40.0	3	60.0	0.84
	HER2 over-expressing	0	0.0	0	0.0	4	100.0	0.22
T	T1	0	0.0	3	42.9	4	57.1	0.74
	T2	2	8.0	8	32.0	15	60.0	0.50
	T3	0	0.0	3	37.5	5	62.5	0.76
	T4	0	0.0	0	0.0	0	0.0	
N	N0	0	0.0	4	36.4	7	63.6	0.67
	N1	1	7.1	6	42.9	7	52.0	0.62
	N2	1	25.0	0	0.0	3	75.0	0.71
	N3	0	0.0	4	36.4	7	63.6	0.67
Grade	low grade	0	0.0	3	100.0	0	0.0	0.04
	intermediate	1	11.1	2	22.2	6	66.7	0.67
	high grade	1	3.6	9	32.1	18	64.3	0.64

DISCUSSION

In 40 cases, fifty percent of cases were ranging 41-50 of age, this is comparable with (Alwan NA, 2010) who studied the demographic characteristics and clinico-pathological presentation of patients in Iraq in 721 patients and found that one third of breast cancer cases were between 40 to 49 [(5)].

In this study majority of the cases were grade II and grade III, 23% and 69% respectively, which is comparable with a study done by Rakha et al. in 2008 who studied the Prognostic significance of Nottingham histologic grade in invasive breast carcinoma in 2219 cases and found that 35.6% and 45.6% grade II and grade III respectively [(6)].

Regarding molecular subtypes, this study shows that 34% were luminal A, 23% luminal B HER2 negative, 20% luminal B HER2 positive, 13% triple negative and 10% HER2 over expressing, this result was comparable with many other studies as Broukhaert et al. who studied prognosis and survival of breast cancer patients in relation to molecular subtypes for 4318 patients and they had quite comparable distributions of breast cancer with decreasing percent with ordered subtypes; 42% for Luminal A, 27% for Luminal B/HER2 negative, 14% for Luminal B/HER2 positive, 11% for triple negative, 7% for HER2 over expressing [(7)].

Our results are also comparable to Minicozzi et al. who studied the role of molecular subtypes in breast cancer in 3381 patients and their results show 56% were Luminal A, 22% Luminal B/HER2 negative, 7% Luminal B/HER2 positive, 10% triple negative and 4% HER2 over expressing [(8)].

While Hennigs et al. in their large cohort study on Prognosis of breast cancer molecular subtypes in routine clinical care for 4102 patients found that luminal A type is most frequent type 44.7% , 31.8% Luminal B/HER2 negative, 6.2% Luminal B/HER2 positive, 12.3% Triple negative, and 5.0% HER2 over expressing [(9)].

Wang et al. worked on Breast Cancer Molecular Subtypes and Ki67 role for the prediction of efficacy and prognosis of neoadjuvant chemotherapy in a Chinese population for 240 patients, they revealed that 25.4% of their cases were luminal A, 52.9% luminal B type, 12.6% HER2 over expressing, and 8.8% were triple negative, the slight difference is due to sample numbers and they also put luminal B as one category [(10)]. In general in all these studies luminal A type constitutes the majority of breast cancer cases and the other types come in sequence with the HER2 over-expressing being the least which is consistent with our finding.

Mitochondrial enzymes expression was correlated with all available clinicopathological parameters and show no significance with any of them apart from a significant correlation between HMGCS2 in cancer associated fibroblasts with low grade tumors (P. value=0.04) which may be in a bigger sample there is a significant correlation with other grades too. So ketone bodies production by stromal cells for feeding the cancer epithelial cells can be correlated with the cancer cell aggressiveness. No similar study on breast cancer was found to compare but Saraon, Punit et al. who worked on prostatic cancer found a significant correlation with the all grades of the tumor [(11)].

This study also shows a significant correlation between ACAT1 stromal intensity and N3 (P. Value=0.01) and a highly significant correlation between HMGCS2 epithelial intensity with N2 (P. Value=0.004). So the ability of cancer associated fibroblasts to use the ketone bodies produced by the cancer epithelial cells is significantly correlated with tumor ability to metastasize especially to regional lymph nodes. These findings are quite stupendous and highly support the concept of tumor microenvironment reflecting the interaction between cancer epithelial calls and cancer associated fibroblast in feeding cancer cells especially those with lymph node metastasis may be before the establishment of its own blood supply.

We conclude that breast cancer can fuel itself with ketone bodies of which the production and utilization is significantly correlated with the tumor aggressiveness and lymph node metastatic ability.

REFERECNES

1. Duffy, Michael J. The biochemistry of metastasis. Advances in Clinical Chemistry. 1996. 32: 135–160.

2. Alyssa M. Dixon and Michael Weichhaus. Breast cancer metabolism: Are ketone bodies energetic substrates. Annual Meeting 2016; April 16-20, 2016; New Orleans, LA

3. Ferrier, Denise R., and Richard A. Harvey. Lippincott's Illustrated Reviews: Biochemistry. Philadelphia: Wolters Kluwer Health, 2014:194

4. Nelson, David L., Michael M. Cox, and Albert L. Lehninger. Lehninger Principles of Biochemistry. 6th ed. New York: W.H. Freeman, 2013: 1105

5. Alwan NA. Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. East Mediterr Health J. 2010 Nov;16(11):1159-64.

6. Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z et al. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol. 2008 Jul 1;26(19):3153-8.

7. Brouckaert O, Laenen A, Vanderhaegen J, Wildiers H, Leunen K, Amant F et al. Applying the 2011 St Gallen panel of prognostic markers on a large single hospital cohort of consecutively treated primary operable breast cancers. Ann Oncol. 2012 Oct;23(10):2578-84

8. Minicozzi P, Bella F, Toss A, Giacomin A, Fusco M, Zarcone M, et al. Relative and disease-free survival for breast cancer in relation to subtype: a population-based study. J Cancer Res Clin Oncol. 2013 Sep;139(9):1569-77.

9. Hennigs A, Riedel F, Gondos A, Sinn P, Schirmacher P, Marmé F,et al. Prognosis of breast cancer molecular subtypes in routine clinical care: A large prospective cohort study. BMC Cancer. 2016 Sep 15;16(1):734.

10. Jiayu Wang, Die Sang, Binghe Xu, Peng Yuan, Fei Ma, Yang Luo. Value of Breast Cancer Molecular Subtypes and Ki67 Expression for the Prediction of Efficacy and Prognosis of Neoadjuvant Chemotherapy in a Chinese Population. Medicine (Baltimore). 2016 May; 95(18): e3518.

11. Punit Saraon, Daniela Cretu, Natasha Musrap, George S. Karagiannis, Ihor Batruch, Andrei P. Drabovich et al. Quantitative Proteomics Reveals That Enzymes of the Ketogenic Pathway Are Associated with Prostate Cancer Progression. Mol Cell Proteomics. 2013 Jun; 12(6): 1589–1601.