Recent Studies & Advances in Breast Cancer

Chapter 5

LKB1 A Novel Therapeutic Target in Breast Cancer- A Way Forward To a Less Aggressive Therapy

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Abstract

Breast cancer is a most common malignancy among females. There has been a number of biomarkers showing therapeutic potential, recently LKB1 gained much attention. LKB1 is an up-regulator of the AMPK pathway and manages cell energy metabolism. It also plays key role as tumour suppressor gene in association with p53. It has been associated with oestrogen receptor too. The activation of AMPK inhibits mTOR which is known therapeutic target in metastatic breast cancers, where everolimus is used. Metformin on the other hand is a hypoglycemic agent which acts through LKB1. The utilization of the metformin as an up-regulator of the LKB1 and AMPK pathway need further exploration as a potential therapeutic target.

1. Introduction

Breast cancer is the most common cancer among females all around the world. Despite the geographical variation it maintains its high rank [1]. It is the second leading cause of death, though there has been significant improvement in the survival rate in the developed countries

owing to the national screening programs and research towards more individualized treatment. In addition to the conventional prognostic markers (ie histological grade, axillary lymph node stage, hormone receptor status) there has been a number of other molecular markers showing their clinical significance. As a result molecular classes have been identified, most commonly used are the five molecular types namely: Luminal A, Luminal B, Basal, HER2 over-expressing and normal like. In these classes both luminal types are hormone receptor positive phenotypes, basal like are hormone receptor negative (also includes triple negative phenotype) while HER2 shows exclusive HER2 positivity regardless of other molecular expressions [2]. Presence of luminal cytokeratins also plays a part in dividing breast cancers into classes. In addition there are other markers including Ki67, p53, Bcl2, EGFR, VEGF and a number of other molecules present in the cells and perform routine duties in normal state as well as in cancer. Mutation of some of these factors lead to cancer development, others play a supportive or preventive role. One of the upcoming therapeutic targets in breast cancer is Liver Kinase B-1 (LKB1). It is a protein kinase encoded by Serine/Threonine kinase -11 (STK11) gene located at the short arm of chromosome 19 at position 13.3 (19p13.3). Upon activation the gene produces LKB1 which is required for the normal growth and function of many cells of the body. So far two isoforms of LKB1 have been identified including LKB1 long and LKB1 short. They have different locations like LKB1 short is mainly fund in testes while LKB1 long is found in other locations of the body. It controls growth by acting as tumour suppressor, and by inducing apoptosis. It also helps in cellular polarization. The LKB1 works in close association with 5'Adenosine-Monophosphate-protein kinase (AMPK) which in turn regulates energy metabolism [3,4]. The mutation of STK11 gene resulting in the abnormal LKB1 causing a number of conditions including cancers. Peutz-jager's syndrome is the caused by LKB1 abnormality [5,6]. PJ syndrome has also been linked with increased risk of many cancers including breast cancer (over 45% patients with PJ syndrome) [7]. The link of LKB1 with AMPK pathway has been studied and shown to be associated with oestrogen pathway and mammalian target of Rapamycine (mTOR) making LKB1 a potential therapeutic target in breast cancer where ER and mTOR inhibitors are available as therapeutic targets. The tumour suppressor effect of LKB1 has been shown to be linked with p53 and bcl2 in managing DNA repair and apoptotic mechanisms. On the other hand pharmacological mechanisms of metformen in association with AMPK pathway makes it an interesting agent to be considered for breast cancer treatment [8].

2. LKB1- AMPK Pathway

5' Adenosin Monophosphate- activated protein kinase (AMPK) is a enzyme comprising of three protein subunits. This pathway is regulated by LKB1 as its main upstream kinase and maintains energy metabolism. In situations of low energy LKB1 inhibits AMPK pathway. The link of LKB1- AMPK also maintains cell polarity including tumour cells thus limiting metastases however in cases of mutated non functional LKB1 causes less optimal growth

suppression and high risk of metastases due to reduced cell polarity[9]. The activation of AMPK also activates p53 which maintains DNA stability, thus suppressing development of tumours, nonethless playing a role in tumour suppressor pathway [10]. Calcium/calmodulin-dependent protein kinase (Ca MKK)has potential to directly stimulate AMPK which is independent of LKB1, while AMP and ATP activated by cellular stress and exercise respectively also activate AMPK. When Leptin receptors are activated by its ligand Leptin it also activates AMPK. Although their simulations are different but they all activate AMPK directly. Once AMPK is activated it stimulates the function of p53 gene, development of endothelial Nitric Oxide Synthase (eNOS). This function is particularly associated with endothelial surface of the vessels. AMPK inhibits Acetyle-CoA Carboxylase which is associated with the function of mitochondria and regulates energy metabolism. AMPK also decreases effects of Transforming growth factor-β (TGF-β) when activated by LKB1. However in LKB1 mutant conditions there has been rise in the circulating TGF and increased susceptibility of the cancer including breast cancer [11]. Figure 1 portrays AMPK pathway.

The study on mouse models and cell lines suggested that LKB1 induces activation of AMPK which in turns locks mTOR, while in situations where LKB1 is knockout or mutant then there is rise in mTOR and increase in the cancer cell growth. Not only increase cancer cell growth but mTOR also make metabolic reprogramming of the cell by shifting it to aerobic glycolysis [12,13]. A summary of the literature commenting on LKB1 –AMPK pathway is given in **Table 1**.

Table 1: summary of the literature exploring 5' Adenosin Monophosphate- activated protein kinase (AMPK) interaction with LKB1

Author	Year	Study design	Results
Mauro L[14]	2018	Cell line study (MCF 7 cells)	Adiponectin induced activation of AMPK pathway is brought about by LKB1. Adeponectin plays inhibitory role in ER negative tumours.
Li NS et al [11]	2016	Human serum samples Animal models	LKB1 activates AMPK – reduces TGF-β* Metformin activates AMPK via LKB1 and reduces cancer development by decreasing TGF-β
Avtanski DB [15]	2015	Cell line study (ie MCF 7 and MDA-MB-468)	1. Leptin is a molecule associated with AMPK and obesity while Honokoil has potential to block leptin 2. Leptin blockage by Honokoil needs LKB1
Andrade Vieira R [13]	2014	Mouse model (LKB-/-NIC)	LKB1 assists in regulating AMPK which then regulates mTOR function, thus abnormality of LKB1 leads to metabolic dysfunction related to AMPK and mTOR
Liu L [16]	2011	Cell line study (MCF7 cells)	Adiponectin increases AMPK via up regulation of LKB1. It then reduces metastatic potential of breast cancer cells
Taliaferro-Smith L [17]	2009	Cell line study (MCF7 and T47D cells)	Adiponetin require LKb1 for activation of AMPK which in turn reduces metatsitic and invasive potential of breast cancer cells

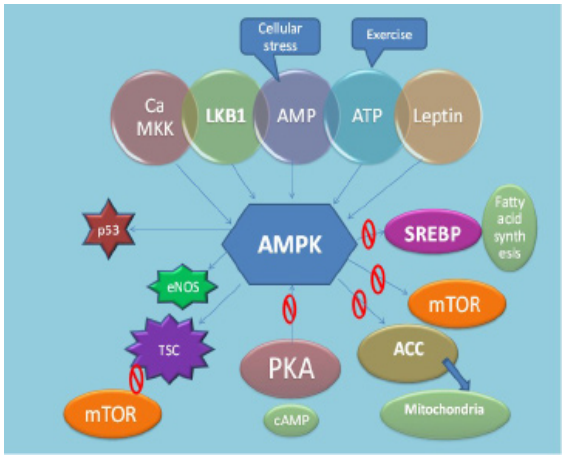

Figure 1: 5' Adenosin Monophosphate- activated protein kinase (AMPK) pathway – activators and regulator mechanism

3. LKB1 Relationship With Oestrogen Receptor (ER)

A recent study on older women showed association of LKB1 over-expression of ER positive early operable primary breast cancer patients who were treated with adjuvant endocrine therapy [18]. In this study role of LKB1 in the oestrogen pathway has been highlighted. Technically the loss of LKB1 (mutant gene) has been linked with development of breast cancer [19]. It was associated with papillary carcinomas in one study and in other it was linked with high grade cancers [20]. Immunohistochemically LKB1 protein is found in the nucleus as well in the cytoplasm. Some studies have evaluated nuclear expression others have considered cytoplasmic expression. However it has not been established yet that the cytoplasmic relocation of LKB1 is a normal phenomenon of its work or it comes out of nucleus in abnormal situations. However, previously conducted study on MCF 7 breast cancer cell lines showed LKB1-ER interaction in the nucleus only[19]. Therefore LKB1 increase the activity of ER in presence of oestrogen by acting as co-activator. Another study focusing on mechanism of adiponectin investigated ER-KB1 interaction, and showed that adiponectin principally worked via AMPK pathway which is in turn activated by LKB1, while ER in presence of adiponectin negatively influences the capacity of LKB1 in relation to the activation of AMPK [14]. The LKB1 has been shown be activated by estradiol, however this activation is dose dependant. The ER signaling of LKB1 in cancer cell lines was observed in the cytoplasm, while in the nucleus LKB1 acts a transactivator of ER. A summary of the present studies is given in **Table 2**.

Table 2: Summary of the literature focusing on relationship ofLKB1 with Oestrogen receptor

Author	Year	Study design	Results
Mauro L [14]	2018	Cell line study (MCF 7 cells)	Adiponectin induced activation of AMPK pathway is brought about by LKB1. Adiponectin plays inhibitory role in ER negative tumours.
Lipovaka Y [21]	2015	Cell line study (T47D cells)	LKB1 upregulated by oestrogen and in turn activates AMPK pathway
Bouchekioua-Bouzaghou K [22]	2014	Cell line study	LKB1 is an important factor for ER cytoplasmic signaling
Linher-MelvilleK [23]	2012	Cell line study (MCF7 cells)	LKB1 mRNA was significantly low in ER+ve cell lines. Treatment of MCF 7 cells with 17-β-estradiol resulted in rise in LKB1 and reduction in ER expression.
Brown KA [24]	2011	Cell line study (MCF7 cells)	LKB1 expression decline in response to treatment with 17-β-estradiol is dose dependant
Nath-Sain S [19]	2009	Cell line study	LKB1 interacts with ERα in the nucleus and enhances ERα transactivation

4. LKB1 as Tumor Suppressor

LKB1 is associated with P53 and blc2 in their activities, thus it has potential role in suppression of cancer development. It is evident that LKB1 mutation in Peutz-Jager's syndrome increases susceptibility to a number of cancers including breast cancer. In addition to its association with p53 and bcl2 it is also linked with adiponectin which played a protective role by causing accumulation of high amount of autophagosomes causing autolysis f the cancer cells. The same study also showed that LKB1 is a major regulator of the autophagolysis induced by adiponectin [25]. Another study on MDA-MB-231 mesenchymal cell lines progressed when LKB1 was upregulated thus it is suggested that presence of LKB1 plays an essential role in the metabolic stress times of the tumour cells [26]. However if LKB1 remains high these tumor cells remain dormant in the circulation with colonizing at metastatic site in contrast if the LKB1 is suppressed then the tumour cells develop metastatic colonies [26]. Another study showing a little contradictory results, however without affecting the concept and stated that it is p53 wild type which activates other associated genes for DNA repair, these genes include PTEN and LKB1 too. In relation to the development of cancer it was reported that the mouse models having knock out LKB1 gene developed breast cancer frequently and cell lines with low LKB1 showed high invasiveness and metastatic potential. While in cases of upregulated LKB1 breast cancer development was not that frequent and if develop it was low grade and low metastatic potential. The relevant literature is summarized in **Table 3**.

Table 3: Summary of literature on LKB1 as a tumour suppressor

Author	Year	Study design	Results
Tang YC [27]	2018	Cell line study	PTEN synthetic- lethal gene include LKB1 were found to be potential drugable targets. PTEN and LKB1 showed many mutually exlusive mutations in cancers.
Chung SJ [25]	2017	Cell line study	Breast cancer cells enter into apoptosis by accumulation of autophagosomes
Pappas K [28]	2017	Cell line study	P53 under normal and low stress conditions regulate a number of other genes including LKB1 and its low expression is associated with increase cancer susceptibility.
Xie B [29]	2017	Cell line study	Benzyle IsoTheoCynate potentiates p53 signaling and anti-tumour effects is brought about by activation of p53-LKB1 axis
Wang YS [30]	2016	Cell line study	LKB1 plays an important role in DNA repair, LKB1 mutant cancer cells are likely to respond to DNA targeting chemotherapeutic drugs
Cheng H [31]	2009	Cell line study	LKB1 show link with p53 function in preventing tumerigenesis.

5. LKB1 Regulation of Metformin Mechanism of Action

A recent study on rats showed anti-tumour activity of the metformin in breast tumours [32]. It further highlighted that the anti-tumour effect is brought about by AMPK pathway. The role of metformin in preventing development of breast cancer was also observed in community based study where there was significantly low rate of breast cancer development in diabetic patients taking metformin as compared to those who were on some other anti-hyperglycemic agents. However in the recent study on rats was conducted where rats were treated with 2-amino-1-methyl-6-phenylimidaz o[4,5-b] pyredine (PhIP) to induced breast tumours and other group of rats was treated with additional metformin. the occurrence of benign tumours was seen in metformin group while majority was malignant in PhIP group [32]. The PhIP group had higher level of markers associated with cellular stress and disturbed serum lipid profile was also observed. This can be explained by the involvement of LKB1 in maintaining cell proliferation, polarity and cell energy metabolism thus preventing its malignant transformation. Thus it can be speculated that metformin up-regulated AMPK which then develop these effects. Theoretically this observation can be translated in clinical terms that even in the presence of major risk factors (such as PhIP in rats) the metformin has potential to reduce development of breast cancer at least in mouse models. Another point merits discussion is the link of AMPK and mTOR, which is an established therapeutic target in breast cancer. mTOR inhibitor (eg Everolimus) is in clinical practice for ER positive metastatic patients who have developed resistance to hormonal therapy. AMPK inhibits mTOR, thus when AMPK is upregulated by activation of LKB1 it in turn inhibits mTOR. Therefore distantly or hypothetically metformin can be linked with mTOR inhibition. **Table 4** summarises the literature on the relationship of LKB1and metformin.

Table 4: Summary of literature on relationship of LKB 1 and Metformin

Author	Year	Study design	Results
Brown KA [33]	2010	Cell line studies	Metformin increases LKB1 expression which activates AMPK pathway and decrease expression of aromatase. Metformin also inhibits nuclear translocationofCRTC2,which is a CREB co-activator and involved in increased aromatase activity
Dowling RJ	2007	Cell line study	Metformin activates AMPK pathway, which then inhibits mTOR

6. LKB1- Metformin- Breast Cancer Future Research Directions

There has been strong evidence that LKB1 regulates AMPK pathway, which then inhibits mTOR and activates p53 which is key DNA repair gene. The involvement of LKB1 in breast cancer development is multi-factorial, by its involvement in oestrogen metabolism, in DNA repair and managing energy metabolism. It has been reported that LKB1 loss is associated with more aggressive tumor biology such as high histological grade, HER2 positive and ER negative status, high Ki67 and VEGF. Its loss was also observed to be associated with poor survival. From the existing literature where most of the studies were carried out on cell lines or some on animal models suggest that upregulation of LKB1not only prevents cancer development but also prevents it spread. Metformin on the other hand is one of the upregulators of the LKB1. It is already in clinical practice for diabetes, therefore it makes metformin an attractive new treatment option. Given that the risk of breast cancer development increases with age so that of the diabetes. Thus metformin may be studied as preventive medicine in diabetic patients in the first place. It may also be studied in combination with adjuvant endocrine therapy in particular luminal B type of breast cancer where patients potentially develop resistance at some stage of the treatment. It was also observed that LKB1 protects DNA thus in cases with its loss there is significant delay in DNA repair and that's the best approach to utilize DNA targeting chemotherapeutic agents. HoweverLKB1 has not shown it independent clinical significance in terms of prognostic factor however there is considerable supporting evidence to take it as predictor factor for use of metformin and DNA targeting chemotherapeutic agents.

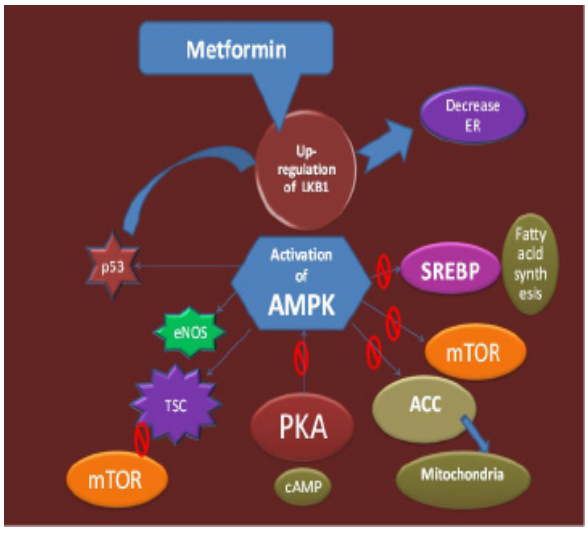

Figure 2: Hypothetical mechanism of action of Metformin on breast cancer cells

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