Maryam SABOUR TAKANLOU

CANCER PATIENTS RETROSPECTIVE ANALYSIS OF *YAP/TAZ/ MST/ LATS* GENE EXPRESSIONS IN INVASIVE DUCTAL CARCINOMA BREAST CANCER PATIENTS

MASTER OF THESIS

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THESIS SUPERVISOR:
Prof. Dr. Gulsah CECENER

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BURSA ULUDAĞ ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ

ETİK BEYANI

Yüksek Lisans tezi olarak sunduğum

"İnvaziv Duktal Karsinom Teşhisi Almış Meme Kanserli Hastalarında *YAP/TAZ/MST/LATS* Gen Ekspresyonlarının Retrospektif Olarak Araştırılması" adlı çalışmanın, proje safhasından sonuçlanmasına kadar geçen bütün süreçlerde bilimsel etik kurallarına uygun bir şekilde hazırlandığını ve yararlandığım eserlerin kaynaklar bölümünde gösterilenlerden oluştuğunu belirtir ve beyan ederim.

Maryam SABOUR TAKANLOU
19.08.2019

III

KABUL VE ONAY SAYFASI

SAĞLIK BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜ'NE

Tıbbi Biyoloji Anabilim Dalı Yüksek Lisans öğrencisi Maryam SABOUR TAKANLOU tarafından hazırlanan "**İnvaziv Duktal Karsinom Teşhisi Almış Meme Kanseri Hastalarında *YAP/TAZ/MST/LATS* Gen Ekspresyonlarının Retrospektif Olarak Araştırılması"** konulu Yüksek Lisans tezi 19/08/2019 günü, ...-..... saatleri arasında yapılan tez savunma sınavında jüri tarafından oy birliği/oy çokluğu ile kabul edilmiştir.

	Adı-Soyadı	**İmza**
Tez Danışmanı	Prof. Dr. Gülşah ÇEÇENER	
Üye	Prof. Dr. Berrin TUNCA	
Üye	Prof. Dr. Feray KÖÇKAR	

Bu tez Enstitü Yönetim Kurulu'nun ..... tarih ve ..... sayılı toplantısında alınan ..... numaralı kararı ile kabul edilmiştir.

Prof. Dr. Gülşah ÇEÇENER
Enstitü Müdürü

TEZ KONTROL ve BEYAN FORMU

19/08/2019

Adı Soyadı: Maryam SABOUR TAKANLOU

Anabilim Dalı: Tıbbi Biyoloji

Tez Konusu: **İnvaziv Duktal Karsinom Teşhisi Almış Meme Kanseri Hastalarında *YAP/TAZ/ MST/ LATS* Gen Ekspresyonlarının Retrospektif Olarak Araştırması**

ÖZELLİKLER	UYGUNDUR	UYGUN DEĞİLDİR	AÇIKLAMA
Tezin Boyutları	☑	☐	
Dış Kapak Sayfası	☑	☐	
İç Kapak Sayfası	☑	☐	
Kabul Onay Sayfası	☑	☐	
Sayfa Düzeni	☑	☐	
İçindekiler Sayfası	☑	☐	
Yazı Karakteri	☑	☐	
Satır Aralıkları	☑	☐	
Başlıklar	☑	☐	
Sayfa Numaraları	☑	☐	
Eklerin Yerleştirilmesi	☑	☐	
Tabloların Yerleştirilmesi	☑	☐	
Kaynaklar	☑	☐	

DANIŞMAN ONAYI
Unvanı Adı Soyadı:
Prof. Dr. Gülşah ÇEÇENER
İmza:

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Meme kanseri kadınlarda en sık görülen kanserdir. Ülkemizde meme kanseri görülme sıklığındaki artış ve görülme yaşındaki düşüş göz önüne alındığında meme kanseri farkındalığı ve tarama programlarının önemi gün geçtikçe daha da artmaktadır. Ayrıca, meme kanseri tedavileri, direnç ve güvenilir biyobelirteç eksikliği gibi birçok sınırlamaya sahiptir. Günümüzde yapılan araştırmalar, Hippo yolağının, hücre çoğalmasının ve apoptozun kontrolünde rol oynadığı gösterilmiş olup, bu yolakta görev yapan genlerde meydana gelen değişikliklerin kanser gelişimi ile ilişkili olduğu ifade edilmektedir. Bununla birlikte, meme kanseri gelişiminde Hippo yolağın düzensizliklerinin moleküler mekanizması ve Hippo yolağı bileşenlerinin klinikopatolojik özelliklerle ilişkisi henüz açıklanmamıştır. Bu çalışmada, invaziv duktal karsinom teşhisi almış meme kanseri hastalarında Hippo yolağında görevli *YAP/ TAZ/ MST/ LATS* genlerinin ekspresyonları retrospektif olarak RT-PCR yöntemi ile araştırıldı ve bu genlerin ifade düzeylerindeki değişiklikler hastaların klinikopatolojik özellikleri ile karşılaştırıldı. Elde edilen bulgular meme kanserli hastaların tümor dokularında *YAP1*, *LATS1* ve *LATS2* ifadelerinin istatistiksel olarak anlamlı oranda (p=0.000; p=0.0004; p=0.0001; sırasıyla) down-regüle edildiğini göstermiştir. Ayrıca, çalışmada Hippo yolağının önemli bileşeni *YAP* mRNA ekspresyonu ile *PR*, grade ve ki-67 indeksi, tümör çapı, metastaz ve lenf nodu arasında anlamlı ilişki olduğu belirlendi. *LATS2* mRNA ifadesindeki farklılığın ise; ki-67, tümör metastazı ve lenf nodu ile istatistiksel olarak anlamlılık gösterdiği belirlendi. Sonuç olarak, Hippo yolağı ile ilgili literatürde yer alan deneysel araştırmalara mevcut tez çalışmasında elde edilen klinik verilerin katkısı ile Hippo yolağının meme kanseri gelişiminde önemli rolü olduğu gösterilmiştir.

Anahtar Kelimeler *YAP*, *TAZ*, *MST*, *LATS*, IDC meme kanseri

ENGLISH ABSTRACT

Retrospective Analysis of *YAP/TAZ/ MST/LATS* Gene Expressions in Invasive Ductal Carcinoma Breast Cancer Patients

Breast cancer is the most frequent cancer type in women. When we consider the increase in its the frequency and the decrease in age of diagnosed patients, we realize that the importance of a screening program and breast cancer awareness is increasing day by day in our country. Also, treatments for breast cancer have many limitations, such as resistance and a lack of reliable biomarkers. Recent studies have also shown that aberrant Hippo pathway functioning can drive tumor formation and breast cancer metastasis due to its critical role in regulating cellular proliferation and organ size. This signaling controls cancer development, cell proliferation, and regulation of apoptosis. However, the detailed molecular mechanism that regulates the Hippo signaling in breast cancer metastasis, and the association of Hippo pathway components levels with clinicopathological characteristics are yet to be fully elucidated. In the present study, the Hippo pathway member levels (*YAP1, TAZ, LATS1/2, MST1/2*) in 100 patients with IDC breast cancer analyzed, and the association of Hippo pathway components levels with survival and clinicopathological characteristics of patients by RT-PCR were assessed. Our study showed that *YAP1, LATS1,* and *LATS2* were significantly (p= 0,000; p= 0,0004; p= 0,0001; respectively) down-regulated in tumor tissues. Also, in this study observed significant association between the expression of low-*YAP* with *PR* status, *HER2* status, tumor grade, Ki-67 proliferation index, metastasis tumors, lymph node, and tumor size. As well as, in the present study demonstrated significant association between the expression of low-*LATS2* with Ki-67 proliferation index, metastasis tumors, and lymph node. In conclusion, studies are shown that the Hippo pathway plays an important role in the development of breast cancer by combining the clinical data obtained from the experimental studies in the literature.

K**eywords** *YAP, TAZ, MST, LATS,* IDC breast cancer

1. INTRODUCTION

Breast cancer is a heterogeneous disease and differs greatly among different patients and even within each individual tumor. Heterogeneity in the expression of established prognostic and predictive biomarkers, hormone receptors, and human epidermal growth factor receptor 2 oncoprotein is the basis for targeted treatment. Invasive ductal carcinoma (IDC) is the most common form of breast cancer, comprising 70% to 80% of all breast cancers. While the current model for breast cancer classification has prognostic value, the lack of a molecular component to the classification scheme limits the ability to predict response to newer targeted therapies. By detecting the expression levels of *ER* and progesterone receptor (PgR), as well as the *HER2* and *Ki-67*, breast cancer was identified into categories with similar clinical implications, including Luminal A, Luminal B, *HER2*-positive, and triple-negative breast cancer (TNBC) subtypes. Recent studies have also shown that aberrant Hippo pathway functioning can drive tumor formation and metastasis due to its critical role in regulating cellular proliferation and organ size.

The Hippo signaling is a conserved mammalian. Mutations and expression of (*YAP*, *TAZ*, *LATS1*, *LATS2*, *MST1*, and *MST2*) promote the migration, invasion, malignancy, and chemotherapy resistance of breast cancer cells and this pathway activation is triggered by contact inhibition and substrate-induced stress that occur when the cellular density reaches a certain threshold. The kinase signaling forms the core of the Hippo pathway and begins with *MST1/2* phosphorylation of *LATS1/2* to form a *LATS1/2-MST1/2* complex, a process assisted by two scaffold proteins *SAV1* and *Mob1*. Activated *LATS1/2* then goes on to phosphorylate downstream effectors *YAP* and *TAZ*. *YAP/TAZ* with binding to a 14-3-3 protein caused to their cytoplasmic

protease degradation and phosphorylation of *YAP/TAZ* results in the inhibition of cell proliferation. These reactions are regulated to control cell proliferation, apoptosis, and differentiation. But, if the upstream signaling kinases are not activated, the hypophosphorylated effector molecules *YAP/TAZ* translocate into the nucleus, and *YAP/TAZ* exerts their oncogenic functions by combining with the transcription factors *TEA* family (*TEA* domain family members, *TEAD*) of transcription factors to promote the expression of transcriptional genes involved in cell proliferation and epithelial-mesenchymal transition (EMT), and cancer metastasis. However, the exact molecular mechanism that regulates the Hippo pathway in breast cancer metastasis, yet to be fully elucidated.

In the present study, the Hippo pathway component levels of 100 patients with IDC breast cancer with a follow-up period of 60 months were analyzed, and the association of Hippo pathway components levels with clinicopathological characteristics of patients were assessed.

2. BACKGROUND

2.1 Cancer and Epidemiology of Cancer

Cancer, among the non-communicable diseases, is the first leading cause of death. It is responsible for an estimated 18.1 million new cases and 9.6 million cancer deaths in 2018 (WHO., 2018). Moreover, among the various cancer subtypes, breast cancer is the second most reported malignancy and the first within women with 2.09 million cases; 1 out of 4 women was diagnosed with breast cancer, with a survival rate greater than 85% (ACS., 2018; SEER., 2018). Annually, about 2 million new cases are diagnosed with breast cancer. The rate of breast cancer is affected by several risk factors, including age, sex, hormonal disorders, family background, lifestyle, population growth, physical activity, and obesity (Hankinson et al., 2004). Indeed, improvements in prevention, early detection, and innovative treatment approaches have significantly increased the number of positive outcomes, even if the heterogeneity of the disease and the limitations of current treatments still make breast cancer a significant concern for public health (Feffer et al., 2018). Cancer is the most frequently diagnosed disease and is also the leading cause of death in Turkey. In Turkey, lung, prostate and urinary bladder cancer are more common in the male population. Breast, thyroid and colorectal cancers appear more in the female population. In Turkey, was estimated which there will be about 22,345 cases of female breast cancer cases in 2018 (Figure 1) and so far there is no enough statistical data for 2019.

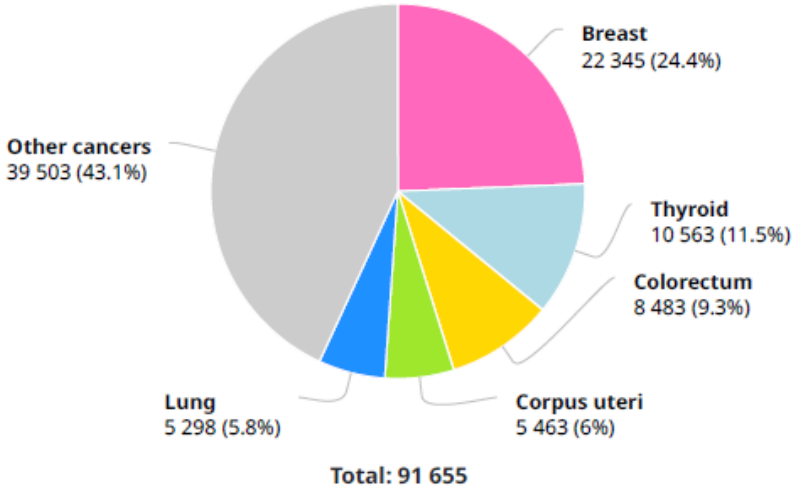


Figure. 1 Number of new cases in 2018, females, all ages. Source: GLOBOCAN 2018.

2.2 Breast Cancer and Historical Classification of Breast Tumors

Breast cancer is the most frequently diagnosed cancer among females, accounting for 25% of all cancer cases worldwide (Torre et al., 2015). Over the past decades, despite substantial efforts made to improve the survival and quality of life, breast cancer remains a deadly threat for patients. For most types of breast cancer, treatment involves surgery, radiation therapy, hormone therapy, chemotherapy, and the latest targeted therapy (Aebi et al., 2011). Up to the present day, multiple targeted drugs have been approved in the treatment of breast cancer by food and drug administration (FDA) that including inhibitors of estrogen receptor (*ER*), aromatase, cyclin-dependent kinase (*CDK*) 4/6, mTOR (rapamycin), and poly (ADP-ribose) polymerase (PARP), and epithelial growth factor receptor (*EGFR*) and human epithelial growth factor receptor 2 (*HER2*)-targeted agents (Alvarez et al., 2010). Additionally, studies about the antitumor effects of many other inhibitors such as inhibitors of vascular endothelial growth factor (VEGF), farnesyl transferase, and fibroblast growth factor receptor (*EGFR*) also show a promising future and have caught attentions as well (Coates et al., 2015). However, the current treatment has its own limitations. First of all, breast cancer subtypes show different responses to systemic therapy, suggesting the treatment should be more specific for each patient (Goldhirsch et al., 2011; Rouzier et al., 2005). Unlike colon cancers, defining the progression of breast cancer has not been possible due to a lack of markers that

define hyperplasia (typical and atypical), carcinoma in situ and invasive cancer. However, breast cancer can be broadly categorized into in situ carcinoma and invasive (infiltrating) carcinoma. Breast carcinoma in situ is further sub-classified as either ductal or lobular; growth patterns and cytological features form the basis to distinguish between the two types. Ductal carcinoma in situ (DCIS) is considerably more common than its lobular carcinoma in situ (LCIS) counterpart and encompasses a heterogeneous group of tumors. DCIS has traditionally been further subclassified based on the architectural features of the tumor which has given rise to five well-recognized subtypes: Comedo, Cribiform, Micropapillary, Papillary and Solid (Connolly et al; 2004; Stingl et al., 2007). While this classification scheme has been a valuable tool for several decades, it relies solely on histology without utilizing newer molecular markers that have a proven prognostic significance (Silverstein et al., 1995). While the routine use of these markers for DCIS has not been accepted by the larger medical community, it is notable that the National Comprehensive Cancer Network has included determination of *ER* status as part of their DCIS workup (NCCN., 2010). This paradigm shift foreshadows the future of molecular medicine that we have only recently begun to appreciate. Similar to in situ carcinomas, invasive carcinomas are a heterogeneous group of tumors differentiated into histological subtypes. The major invasive tumor types include infiltrating ductal, invasive lobular, ductal/lobular, mucinous (colloid), tubular, medullary and papillary carcinomas. Of these, infiltrating ductal carcinoma (IDC) is, by far, the most common subtype accounting for 70–80% of all invasive lesions (Li et al., 2005). IDC is further sub-classified as either well-differentiated (grade 1), moderately differentiated (grade 2) or poorly differentiated (grade 3) based on the levels of nuclear pleomorphism, glandular/tubule formation and mitotic index.

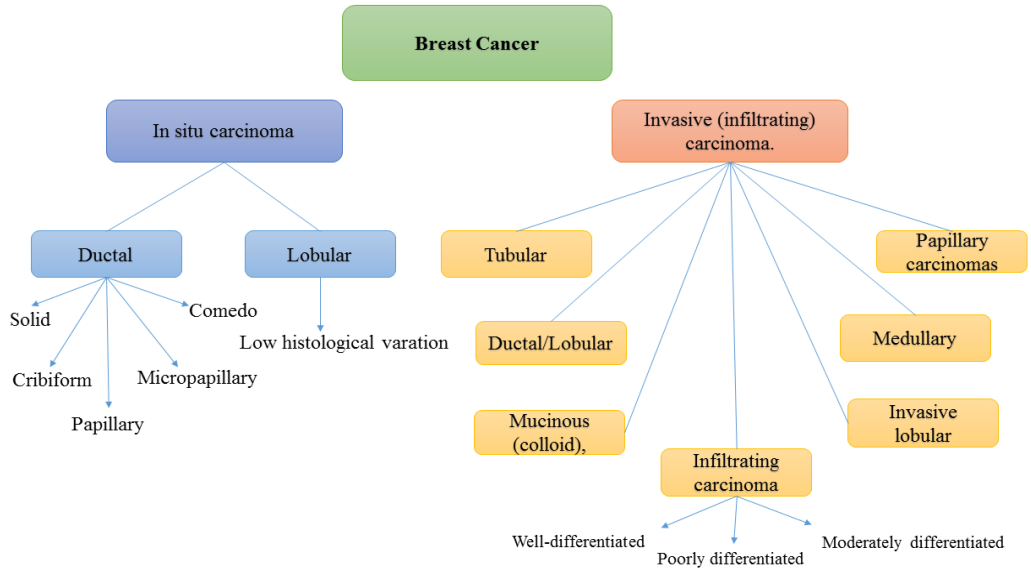


Figure. 2 Histological classifications of breast cancer subtypes (Stingl et al., 2007)

2.3 Breast Cancer Subtypes

While the current model for breast cancer classification has prognostic value, lack of a molecular component to the classification scheme limits the ability to predict response to newer targeted therapies. By detecting the expression levels of ER and progesterone receptor (PgR), as well as the *HER2* and *Ki-67*, breast cancer was identified into categories with similar clinical implications, including Luminal A, Luminal B, *HER2*-positive, and triple-negative breast cancer (TNBC) subtypes (Parker JS et al., 2009). More recently, a new subtype classified as claudin-low has also been identified (Figure 3). These molecular subtypes of cancer were identified by microarray-based gene expression analysis and unbiased hierarchical clustering. Notably, the molecular subtypes display highly significant differences in the prediction of overall survival, as well as disease-free survival with the basal-like/triple-negative (*ER- /PR- /ErbB2-*) subtype having the shortest survival. Furthermore, this molecular classification was able to stratify the *ER+* population into several subtypes that, again, demonstrated a difference in patient survival. This is significant because even though clinical assessment of IDC utilizes *ER*, *PR* and *ErbB2* status, these markers did not allow separation of the two distinct *ER+* subtypes (i.e., Luminal A and Luminal B) that have very different clinical outcomes

(Anderson et al., 2014; Dieci et al., 2014; Malhotra er al., 2010). Among them, *TNBC* shows a relatively poor prognosis, while the Luminal and *HER2*-positive subtypes respond sensitively to endocrine therapy and targeted therapy (Brenton JD et al., 2005). Secondly, drug resistance, especially to monotherapy, has limited the curative efficacy, resulting in a low response rate. Thirdly, the lack of reliable indicators for most of the targeted agents makes it a challenge to select doses and predict the prognosis of patients (Duffy et al., 20017). Therefore, identifying novel breast cancer therapeutic targets, revealing the mechanisms underlying drug resistance, discovering new biomarkers and developing rational combinations of targeted therapy remain urgent and important.

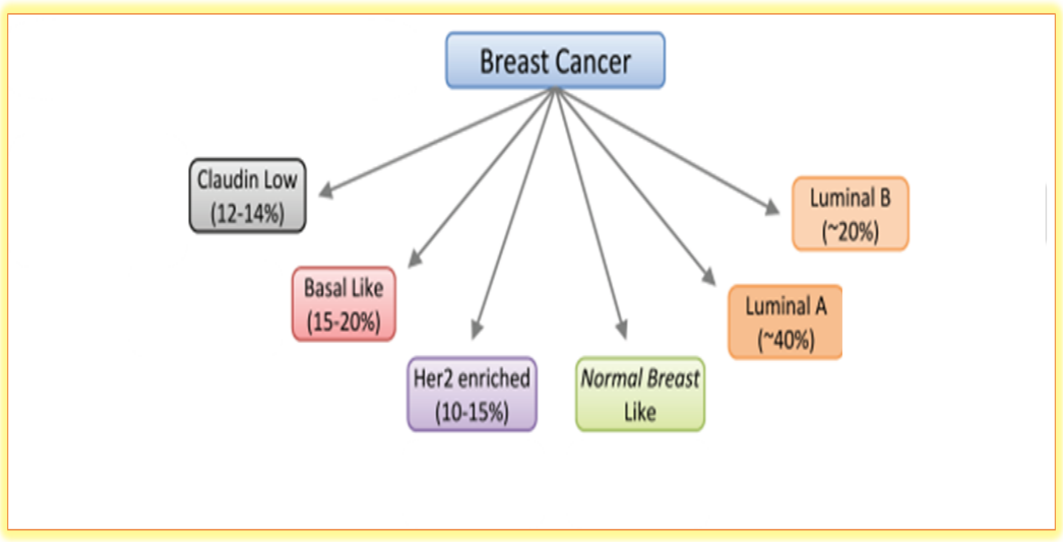


Figure. 3 Molecular classifications of breast cancer. This classification is based on the intrinsic molecular subtypes of breast cancer identified by microarray analysis of patient tumor specimens (Dieci et al., 2014).

2.4 Important Drivers of Breast Cancer

There are a host of different factors that can promote the development of breast cancer. Loss-of-function mutations in tumor suppressor genes or gain-of-function mutations in proto-oncogenes can lead to uncontrolled cellular proliferation and are common hallmarks of cancer (Meric-Bernstam et al., 2014). Tumor suppressors are involved in a multitude of different regulatory processes, including

cell cycle regulation, apoptosis, and DNA repair. Functional tumor suppressor genes are essential to prevent abnormal cells from proliferating, and mutations in these genes can lead to deregulation of the cell cycle, uncontrolled cell growth in the presence of damaged DNA, and ultimately cancer (Meric-Bernstam et al., 2014). The most commonly known loss-of-function mutations of tumor suppressor genes in breast cancer include *TP53*, which codes for *p53*, a critical cell cycle regulator, and *BRCA1*/*BRCA2*, which are both DNA repair genes (Lee et al., 2010). Forty percent of breast cancer cases contain a mutation in *TP53* while *BRCA1* and *BRCA2* mutations account for 25% of hereditary breast cancers (Lippman., 2015). Oncogenes arise from proto-oncogenes through changes in the genetic code, such as chromosome rearrangement, gene duplications, or mutations in base pairs in coding sequences (Morin et al., 2015). Oncogenes promote cell proliferation, growth, differentiation, and survival and include growth factors/mitogens, receptor tyrosine kinases, serine/threonine kinases, regulatory GTPases, and transcription factors (Morin et al., 2015). Commonly mutated oncogenes in cancer include: oncogenic c-terminal cyclin D1 (*CCND1),* which codes for Cyclin D1, an important cell cycle regulator; phosphoinositide-3-kinase *(PI3KCA)* which codes for a *PI3K*, a kinase that promotes growth, proliferation and survival; *ERBB2*, which codes for a receptor tyrosine kinase; and *MYC*, a transcription factor promoting proliferation (Lee et al., 2010). Recent studies have also shown that aberrant Hippo pathway functioning can drive tumor formation and metastasis due to its critical role in regulating cellular proliferation and organ size (Aragona et al., 2013; Low et al., 2014). In recent years, the inactivation of the Hippo tumor suppressor pathway has come to the forefront as a recognized occurrence in human breast cancers.

2.4.1 The Function of the Hippo Signaling Pathway

Hippo signaling pathway began with the discovery of the Wts gene in drosophila melanogaster; and after discovered the homolog of the Wts gene in the human. A highly conserved signal transduction pathway that plays important roles of cellular proliferation, growth and survival (Ganem et al., 2007; Moroishi et al., 2015) organ size control, tissue regeneration, immune response, stem cell function and tumor suppression (Halder et al., 2011; Taha et al., 2018) in cancer-associated

cellular functions (Harvey et al.,2013; Johnson et al., 2014; Moroishi et al., 2015) (Figure 4). Although originally discovered through genetic screening of tumor suppressor genes in Drosophila melanogaster, the Hippo pathway is important in human biology and has broad implications in disease etiology, gaining recognition particularly for its role in cancer (Ganem et al., 2007; Morioshi et al., 2015). The upstream components contain *FAT4* (Fat homology), *FEMD6* (Ex homology), *Mer* (*NF2*, *Mer* homology), and *DCHS1/2* (Dachsous homology). The kinase signaling consists of *MST1/2* (Hpo orthologs), *SAV1* (Sav orthologs), *LATS1/2* (*Wts* orthologs), and *Mob1* (Mats orthologs). The main effectors of the component in the Hippo pathway are *YAP* (yes-associated protein, a yes-related protein) and *TAZ* (transcription co-activator with a *PDZ* binding motif, also called WWTR1; homologs of Yki). The transcription-related parts of the Hippo signaling pathway include *TEF/TEAD1-4* (Sd orthologs), *CTGF* (connective tissue growth factor), *AREG* (amphiregulin), and *Gli2*. The main members of Hippo pathway including *MST1/2*, *SAV1*, *LATS1/2*, in the mammals and *Mob1* which are each activated by phosphorylation to form compounds that transmit apoptotic signals (Zhao et al., 2010). *MST1/2* and *LATS1/2* are serine/tyrosine protein kinases, and *SAV1* and *Mob1* act as protein activators. *YAP* and *TAZ* are the main transcription co-activators of the Hippo signaling pathway. Upstream regulators of the Hippo signaling pathway and the two molecules in this pathway (*LATS1* and *MST1*) have been found to function as tumor suppressor genes. The Hippo signaling pathway is a conserved pathway and important role in regulating cell proliferation, tissue homeostasis, and organ size (Barry ER et al., 2013; Moroishi T et al., 2015; Piccolo S et al., 2014; Varelas., 2014; Yu et al., 2014). The Hippo pathway has a role in maintaining the balance between cell proliferation and apoptosis, its dysregulation is strongly associated with the occurrence and development of human tumor growth and carcinogenesis (Figure 4) (Atkins et al., 2016).

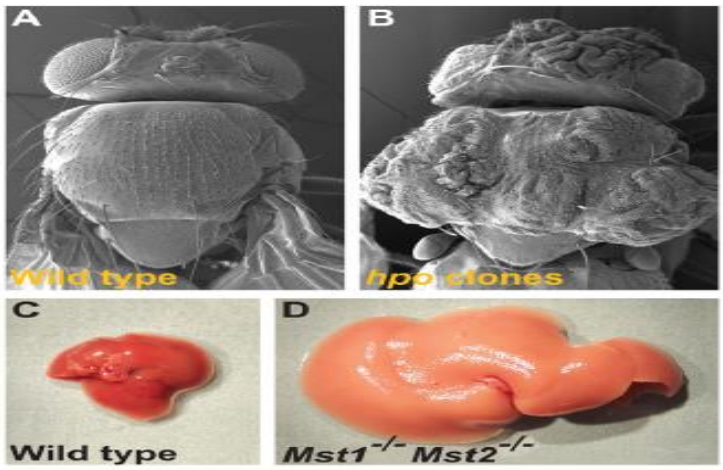


Figure. 4 Hippo mutant phenotypes in flies and mice. (A, B)Scanning electron micrographs of (A) a wild-type fly and (B) a fly with clones of cells homozygous mutant for hippo that exhibit overgrowth of the adult cuticle. (C) A mouse liver at 2 months of age from a wild-type animal and (D) a liver at 2 months of age from a mouse mutant in which both *Mst1* and *Mst2* (*STK3* and *STK4*), two mammalian Hippo homologs, have been conditionally inactivated in the developing liver. The double null Mst1/2 mutant liver is overgrown owing to an increase in cell numbers (Halder et al., 2011).

2.4.2 The Hippo Signaling and it's the Upstream Regulations

The Hippo pathway an intricate network that can be influenced by a host of other factors. Activators of the Hippo pathway inhibit cell growth and promote apoptosis and include metabolic stressors (oxidative or energetic), cytoskeletal defects caused by detachment, contact inhibition, or growth factor deprivations, and cytokinesis failure. The studies has shown that cell-cell contacts, adhesion and apical-basal polarity proteins, mechanical cues from neighboring cells and the extracellular matrix, as well as various signals acting through other signaling pathways, have all been identified as regulators of the localization and phosphorylation of *YAP/TAZ* through *MST1/2* or *LATS1/2*, while some of them regulate *YAP/TAZ* independent of the canonical Hippo pathway (Figure 7) (Ganem et al., 2014; Hansen et al., 2015; Kim et al., 2015; Wennmann et al., 2014). Inhibitors of the Hippo pathway promote cellular proliferation and include osmotic, endoplasmic reticular and mechanical stressors as well as DNA damage (Hansen et al., 2015). The Hippo cascade appears to consistently respond to changes in its cellular cytoskeletal network. When a cell expands or stretches, the Hippo pathway is inactivated, promoting nuclear accumulation of *YAP* and *TAZ*. When a cell is compressed, the Hippo pathway is on and prevents *YAP/TAZ* accumulation in the nucleus and cell growth (Dupont et al., 2011; Yu et al., 2013). Complete disruption

of the cytoskeleton, such as through treatment with Latrunculin A, an actin polymerization inhibitor, results in Hippo pathway activation, which then sequesters *YAP* in the cytoplasm (Thomasy SM et al., 2013). Hippo pathway activity can also be modulated through changes to the actin cytoskeleton induced by tetraploidy, glucose deprivation, serum starvation, contact inhibition and loss of cell adhesion, which are also, not coincidentally, key players in cancer progression (Ganem et al., 2014; Hansen et al., 2015).

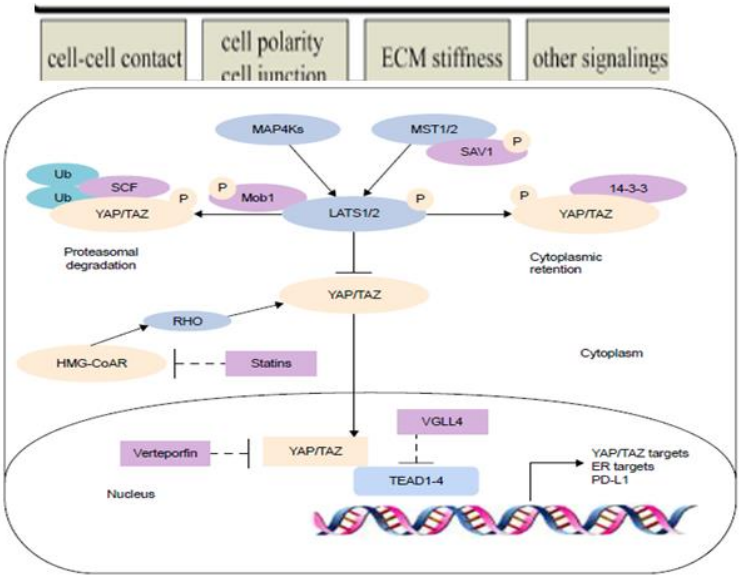


Figure. 7 Upstream regulators of the hippo pathway

YAP and *TAZ* likewise respond to external stresses evoked by these cellular microenvironments. Hyperosmotic stress induces tyrosine phosphorylation of *TAZ* by the *ABL* kinase, which facilitates the interaction between *TAZ* and nuclear factor of activated T cells 5 *(NFAT5)* to inhibit *NFAT5* function in osmoregulatory transcription. *YAP/TAZ* are activated by interstitial flow-driven shear stress and promote osteogenic differentiation of mesenchymal stem cells. *Rho GTPase* appears to be involved in this regulation, although the precise mechanism remains unknown. Oxidative stress evoked by ischemia and reperfusion in the mouse heart activates the Hippo pathway to antagonize a functional *YAP–FOXO1* complex, leading to enhanced oxidative stress-induced cell death. By contrast, hypoxia deactivates the Hippo pathway by destabilizing *LATS2* through Seven in absentia homolog 2 *(SIAH2)* ubiquitin-ligase-induced degradation. Energy stress, such as inhibition of

glucose metabolism and ATP production, induces *AMP*-activated protein kinase *(*AMPK*)*-mediated phosphorylation of Angiomotin-like 1 *(AMOTL1)* to stabilize and increase *AMOTL1*, which in turn stimulates *LATS* (Figure 8) (Hansen et al., 2015).

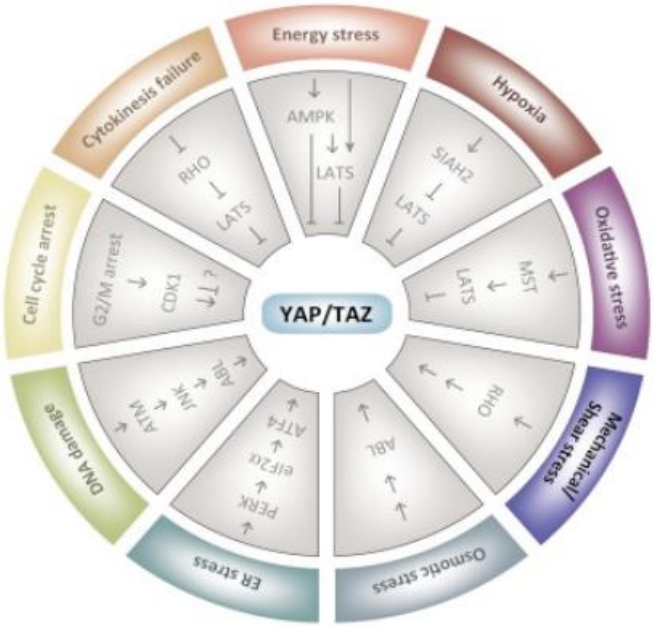


Figure. 8 Examples of the Upstream Regulators of the Hippo Pathway.
YAP and *TAZ* serve as the effectors of the Hippo pathway. These two transcriptional coactivators bind to *TEAD* and are responsible for promoting growth and survival. The Hippo pathway is regulated by a plethora of different factors, many of which have yet to be defined. Some of the possible upstream regulators are shown in this figure. Adapted from (Hansen *et al*. 2015).

2.4.3 Role of Hippo Signaling in Breast Cancer

In women, breast cancer is one of the most common malignant tumors. The molecular pathogenesis of breast cancer has been heavily scrutinized to obtain a deeper understanding of how to more effective treatment (Figure 9). The Hippo components have a crucial role in metastatic breast cancer (Bos et al., 2009). Hippo signaling plays a role in breast cancer bone metastases. *HER3*-phosphorylated in Tyr1307 is able to methylate *MST1* at the Lys59 site, cause the activation of *YAP/TAZ* in breast cancer cells, which promotes bone metastasis (Li et al., 2017). *TAZ* expression significantly in bone metastasis was higher in comparison with primary tumors. Hypoxic states are characterized by the presence of hypoxia-inducible factor *(HIF)-1α. HIF-1α* interacts with *TAZ* to induce breast cancer bone metastasis in a hypoxic microenvironment (Bartucci et al., 2015; Bendinelli et al.,

2013; Xiang et al., 2015). The studies have been found that *HIF-1α* to be largely involved in the EMT in metastasis, and this molecule has been shown to be regulated by interactions between EMT pathway and Hippo pathway factors (Maroni P et al., 2015). Dysregulation in the Hippo signaling pathway also seems to confer enhanced chemoresistance to cancer cells. Cultured breast cancer stem cells show increased chemoresistant properties when compared with differentiated, non-tumor breast cancer cells (dBCCs). *TAZ*-overexpressed in dBCCs enhanced neoplastic transformation and enhanced migratory activity. Inversely, the deletion of *TAZ* in breast cancer stem cells severely impeded the formation of metastatic colonies and decreased chemoresistance (Bartucci et al., 2015). Also, *TAZ*-overexpression can enhance chemoresistance to taxols in the MCF10 breast cell lines. As well as, decreased *TAZ* expression seems to downregulate the sensitivity of *MDA-MB-231* breast cell line to chemoresistance such as taxols (Lai et al., 2011). Decreased the sensitivity of tamoxifen is related to low *YAP* expression and is associated with the clinical results in luminal A subtype of breast cancer (Lehn et al., 2014). Also, the *MST1/2*, and *LATS1/2* act as tumor suppressors that regulate *YAP* phosphorylation (Arash et al., 2017; Hay et al., 2003). Further studies have shown that the reduced expression of *LATS1* or *LATS2* mRNA in breast cancer tissues cause to increased tumor size and lymph node metastasis and is negatively associated to the presence of *ER* and *PR* receptors (Visser et al., 2010). The depletion or mutation of *LATS1/2* cause to an aggressive breast cancer phenotype that displays increased invasive ability. Therefore, *LATS1/2* may be a novel target for anticancer treatment in breast cancer (Cooper et al., 2017). The role of *MST* in breast cancer metastasis less is known. Studies suggest that high *MST1* expression in MCF-7 breast cancer cells can decrease cell proliferation and initiation cell apoptosis (Luo et al., 2011). Interestingly, the potential carcinogenicity of *YAP* is associated with its hypophosphorylated state, which can increase the expression of growth factors and apoptosis-inhibiting factors. Inhibition of Hippo signaling results in *YAP* hypophosphorylation and its nuclear localization. In the nucleus, *YAP* binds to transcription factors to increase the expression of cyclin D1. However, cyclin E expression does not change. Overexpression of *YAP* in MCF10A breast cell line can induce EMT, which is associated with the upregulation of fibronectin, vimentin, and

N-cadherin and the downregulation of E-cadherin and occludin. The activation of *AKT* and *ERK* increases the ability of *YAP* to promote the proliferation of MCF10A cells in the absence of Epidermal growth factor (*EGF)* (Overholtzer et al., 2006). Cells grown at high density were less effective compared with low cell density at invading an endothelial cell monolayer. Treatment with verteporfin and *YAP* knockdown induced downregulation of the cytokines *IL6, IL8,* and *CXC1, CXC2,* and *CXC3* by inhibiting the activity of *YAP* and consequently inhibited vascular invasiveness of breast cancer cells (Sharif et al., 2015). These data suggest that altered growth density of breast cancer cells regulates their vascular invasion by *LATS1-YAP* in the Hippo pathway. In addition, *TAZ* expression is positively associated with EMT, tumorigenesis, tumor migration, and invasion in breast cancer cells (Lei et al., 2008; Mi et al., 2015). *TAZ* is highly expressed in Hs578T, BT-549, and *MDA-MB-435S* invasive breast cancer cell lines and moderately expressed in *MDA-MB-231* cells (Chan et al., 2008). The studies were found that Twist upregulated the expression of *PAR1*, would inhibit *TAZ* phosphorylation and increases *TAZ* activity. *TAZ* depletion suppressed transendothelial invasion of T47D breast cancer cells. In contrast to its oncogenic activity of *YAP/TAZ* in the development of breast cancer and tumorigenesis, there is a school of thought that believes that *YAP/TAZ* may also possess crucial tumor-suppressive role in human oncogenesis (Wang et al., 2014; Wang et al., 2016). Immunohistochemical assay of breast tissues revealed significant nuclear *YAP* expression in normal breast tissues, with *YAP* expression being low or even absent in cancer tissues. *YAP* expression is weak in luminal epithelial cells and also suggested that reduced expression of *YAP* is negatively associated with the presence of estrogen and progesterone receptors in invasive breast carcinomas. Therefore knockdown of *YAP* promotes cell migration, invasion, and tumor growth (Jaramillo-Rodriguez et al., 2014; Tufail et al., 2012; Yuan et al., 2008). *YAP1* interacts with the *p53* family member *p73* to mediate the nuclear stability of *p73*, which leads to the expression of apoptotic genes (Matallanas et al., 2007). In addition, research examining the relationship between the expression of Hippo pathway factors and clinical outcomes of breast cancer hints toward their potential to function as predictive clinical biomarkers in breast cancer patients. Immunohistochemistry investigations in 69 breast cancer samples revealed that

75.4% of clinical breast cancer samples demonstrated elevated *YAP* expressions, with 29% of these cases showing high expressions of *YAP*. However, 24.6% of these breast cancer samples demonstrated no *YAP* expression. It has been suggested that overexpression of *YAP* correlates with tumor formation and growth (Wang et al., 2012). Immunohistochemistry analysis of *TAZ* expression in 640 invasive breast carcinoma samples that comprised estrogen and progesterone receptor-positive (*ER+/PR+*), *HER2*-positive and TNBC tumors suggested that *TAZ* expression was significantly associated with the TNBC phenotype (60.5% *TAZ*-positive, *P*,0.001). Similarly, other studies have reported high *TAZ* expression in the *HER2*-positive and TNBC (Bartucci et al., 2015; Kim et al., 2014; Lehn et al., 2014; Min Kim et al., 2012; Vici et al., 2014). *TAZ* was highly expressed in 90% of breast carcinomas with EMT. An increased proportion of patients whose tumors strongly expressed *TAZ* experienced higher rates of tumor recurrence and worse survival outcomes. *YAP/TAZ* acts as an oncogene in different subtypes of breast cancer (Díaz-Martín et al., 2015). The risk of residual disease in *HER2*-positive or TNBC patients after receiving neoadjuvant therapy has been shown to be related to the subcellular location of both p*MST1/2* and *TAZ*. Patients with strong *TAZ* and *MST1/2* nuclear expressions showed poorer clinical outcomes after neoadjuvant therapy. Conversely, the colocalization of cytoplasmic p*MST1/2* and *TAZ* is likely to be a protective factor in the Hippo pathway. Therefore, *MST1/2* may function as a potential predictive biomarker in *HER2*-positive and TNBC patients (Ercolani et al., 2017). The expression of *TAZ/YAP* was associated with inferior survival in male breast cancer patients (Di Benedetto et al., 2017).

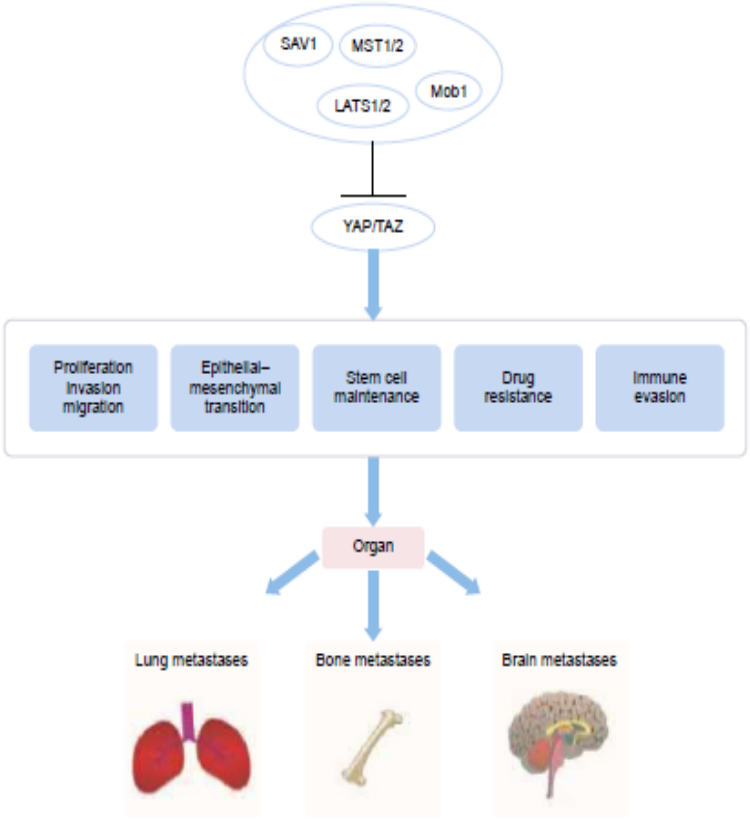


Figure. 9 The Hippo pathway regulates organ metastases (Changran et al., 2018)

2.4.4 Role of *YAP* in Cancer Pathogenesis

The Yes-associated protein (*YAP1*) is a potent growth promoter and was discovered in 1994, and evidence indicates that play important role in pathogenic mechanisms of cancer including tumorigenesis, metastasis, and drug resistance (Sudol et al., 1994; Zanconato et al., 2016). *YAP*1 is located on the 11q22 amplicon (Lamar et al., 2012). The transcriptional coactivator Yes-associated protein (*YAP*) is a major regulator of organ size and proliferation invertebrates. As such, *YAP* can act as an oncogene in several tissue types if its activity is increased aberrantly motif. *YAP1* contains a *TEAD* binding domain necessary for activation of the *TEAD* transcription factors, which upon aberrant activation leads to increased cell growth and proliferation, ultimately resulting in tissue overgrowth (Wu et al., 2008). Studies shown that increased *YAP/TEAD* activity plays a causal role in breast cancer progression and metastasis and *YAP1* to function as an oncogene in breast cancer in many other cancer forms such as ovarian, lung and esophageal squamous cell carcinoma, overexpression of *YAP1* is

correlated to a worse outcome (Lamar et al., 2012). Despite these reports pointing to *YAP1* as an oncogene, the role of *YAP1* in breast cancer is far from clear. Researchers reported that stable downregulation of *YAP1* in breast cancer cell lines resulted in the protection of anoikis, promotion of anchorage-independent growth and increased migration and invasion. *YAP1* depletion resulted in increased tumor growth in nude mice, altogether suggesting a tumor-suppressive function of *YAP1* in breast cancer (Muramatsu et al., 2011; Yuan et al., 2008).

2.4.5 Role of *LATS 1/2* in the Breast Cancer

LATS1 (Large Tumor Suppressor) is serine/threonine (ser/thr) kinase of the AGC kinase family and a novel tumor suppressor gene that is mutated or down-regulated in a variety of human cancers. *LATS1* is involved in tumorigenesis by either inducing apoptosis or negatively regulating cell proliferation, genetic stability, cell migration and metastasis (Visser et al., 2010; Yang et al., 2001). And also has been identified as a central player of the emerging Hippo signaling pathway that was originally discovered in Drosophila and plays important roles in various biological processes such as tumorigenesis, organ size control, stem cell differentiation and renewal, drug resistance, and neuronal dendrite growth and tilling (Lai et al., 2011; Tapon et al., 2012). In this pathway, ser/thr kinases and tumor suppressors *Mst1/2* (mammalian homolog of Drosophila Hippo) and *LATS1/2*, and the transcriptional co-activator and oncoprotein *YAP* and its paralog *TAZ* are the core components. *Mst1/2* phosphorylates and activates *LATS1* and its homolog *LATS2*, which subsequently phosphorylates and inhibits *YAP* and *TAZ* by preventing them from translocating to the nucleus (Badouel et al., 2011; Lei et al., 2008; Lai et al., 2011; Zhao et al., 2011). In recent years, the *LATS1* and *LATS2* Hippo pathway kinases have become the focus of intense research (Furth et al., 2017). Classically, *LATS1* and *LATS2* are viewed as redundant paralogs that phosphorylate and inactivate the transcriptional cofactors *YAP* and *TAZ* (Moroishi et al., 2015). Both *LATS* is downregulated in human breast cancer (Furth et al, 2015), and both have recently been implicated in modulating *ER* protein stability (Britschgi et al, 2017). For instance, *LATS1* knockout mice are highly sensitive to carcinogens and display pituitary dysfunction (St John et al, 1999), whereas conditional *LATS1* knockout results in metabolic defects, such as fatty liver disease

(Aylon et al, 2016). Metabolic control is key to tumor suppression, reflecting the need for tumor cells to adapt their metabolism to support rapid growth. *ER*+ tumors often have increased fatty acid transport and elevated levels of short- and medium-chain fatty acids (Tang et al, 2014), which may affect their metabolic state, in part by regulating the activity of the nuclear (peroxisome proliferator-activated receptor γ) PPARγ (Liberato et al, 2012). This suggests a key role for PPARγ in luminal breast cancer (Zhou et al, 2009). Activation of PPARγ alters the expression of a large set of target genes, affecting adipogenesis, lipid metabolism, inflammation, and metabolic homeostasis (El Akoum et al., 2014). Furthermore, PPARγ activation can exert antiproliferative effects in a variety of cancer types, including breast cancer (Kersten et al, 2000). A study showed that a *LATS2*-associated gene expression pattern is specifically down-regulated in luminal B breast cancer. Deletion of *LATS1* in the mouse mammary gland results in increased luminal B tumorigenesis and metabolic rewiring of the tumor cells. Conversely, *LATS2* stimulates PPARγ signaling and promotes the death of luminal B-derived cells. In contrast, deletion of *LATS1* reprograms luminal B tumors towards basal-like characteristics. Concordantly, low *LATS1* correlates with increased resistance to hormone therapy (tamoxifen). Thus, each *LATS* paralog exerts distinct tumor-suppressive effects in the context of breast cancer, in a subtype-specific manner (Furth et al., 2018).

2.4.6 Role of *TAZ* in The Breast Cancer

In mammals, *TAZ* is a WW domain-containing molecule that is located at chromosome 3q23 that functions as a transcriptional co-activator by binding to proline-proline-any amino acid-tyrosine (*PPXY*) motifs present on transcription factors and is normally expressed highly in heart, lung, kidney, and placenta. *TAZ* also binds to the regulatory 14-3-3 proteins. 14-3-3 proteins bind to serine/threonine-phosphorylated residues in a context-specific manner and bind and regulate key proteins involved in intracellular signaling, cell cycling, apoptosis, and transcription regulation. *TAZ* binds 14-3-3 proteins when phosphorylated on four specific serine residues (S66, S89, S117, S311), serine 89 being the most important. Phosphorylation results in *TAZ* being exported out of the nucleus to the cytoplasm. *TAZ* also contains a post-synaptic density, Drosophila disc large tumor suppressor, and zonula occludens-1 *(PDZ)*-binding motif in

the C-terminus that localizes *TAZ* to discrete nuclear foci and is vital for *TAZ*-stimulated gene transcription (Imajo et al., 2012; Grzeschik et al., 2010). *TAZ* has been identified as an oncogene that plays a critical role in the migration, invasion, and tumorigenesis of breast cancer cells. It is conspicuously overexpressed in human breast cancer tissues from patients in which its expression levels generally correlate with the TNBC diagnosis and patient prognosis. Overexpression of *TAZ* in low-expressing MCF10A non-tumorigenic mammary cells leads to the acquisition of a spindle-shaped morphology and increases migratory and invasiveness (Bartucci et al., 2015; Díaz-Martin et al., 2015; Siew et al., 2008). *TAZ* has been implicated in breast cancer-associated metastatic bone disease, partly through its interaction with hypoxia-inducible factor-1 (Bendinelli et al., 2012). Recent studies show that *TAZ* is required for sustaining self-renewal, tumor-initiation capacities and metastatic activity (Cordenonsi et al., 2011; Diaz-Martin et al., 2015). Besides *TEADs*, *TAZ/YAP* can bind to other transcriptional factors, such as the krueppel-like factor 5 (*KLF5*) and transforming growth factor (*TGF*)-activated SMAD Family Member 2/3 (*SMAD2/3)*. The overexpression of *YAP* could upregulate *KLF5* protein levels and mRNA expression levels of its downstream target genes including *FGFBP1* and *ITGB2* that promote breast cancer cell proliferation and survival (Zhi et al., 2012); the interaction between *TAZ/YAP* and *SMAD2/3* regulates novel targets such as *NEGR1* and *UCA1* that are necessary for tumorigenic activity in metastatic breast cancer cells. *YAP* function is also required for cancer-associated fibroblasts to promote matrix stiffening, cancer cell invasion and angiogenesis (Calv et al., 2013; Hiemer et al., 2014).

2.4.7 The Roles of the Hippo Pathway in Therapeutic Drug Resistance

Advances in cancer therapies have improved patient survival in many types of cancer, but resistance against therapies crucially limits the opportunity of complete tumor remission or further survival improvement. Malignant tumors are based on complex, redundant, and heterogeneous survival mechanisms that prevent tumor death by single pathway blockade (Holohan et al., 2013). The most attractive therapeutic target is the essential oncogene *YAP* as the terminal protein of the Hippo pathway. The inhibition of *YAP* in various cancers is of interest as an anticancer

therapeutic strategy. The therapeutic effect of a *YAP* inhibition is mostly based on genetic (knock-out) studies with mice which demonstrate that the heterozygosity of *YAP* represses cancer development. For instance, colon cancer development in *Sav1* and *MST1/2*-deficient colons was repressed by a *YAP* knock-out or heterozygosity (Cai et al., 2010; Zhou et al., 2011). As molecular alterations of cancer have been extensively profiled by recent genomic and proteomic technologies, researchers now focus on the development of anti-cancer drugs targeting recurrently mutated oncogenic drivers. Although several targeted therapy agents against major oncogenic drivers have been developed, the effectiveness of the agents varies widely because of innate and acquired resistance. Remarkably, recent studies have demonstrated that *YAP* can functionally substitute for oncogenic *KRAS*, which is one of the most common drivers of human cancer. The relapsed tumors acquired *YAP* amplification, and elevated *YAP-TEAD2* activity was shown to enable bypass of oncogenic *KRAS* activity through upregulation of cell cycle and DNA replication genes. Imply that *YAP/TAZ* activation may induce resistance to targeted anti-cancer agents specific for *RAS* signaling pathways. The MAPK pathway (*RAF–MEK–ERK*) is a central node in regulating cell proliferation and survival, and oncogenic mutations in MAPK components, including *BRAF*, constitutively activate cell proliferation. *BRAF* inhibitors and *MEK* inhibitors have proved effective in treating *BRAF* mutant melanoma and NSCLC. However, acquired resistance occurs in the majority of patients in a short time period after initial tumor shrinkage (Chapman et al., 2011; Robert et al., 2015). *YAP* serves as a parallel survival input to inhibit apoptosis upon *RAF* and *MEK* inhibitor treatment, and *YAP* suppression significantly enhances sensitivity to *RAF* or *MEK* inhibitors. Therefore, seem combined *YAP* and MAPK inhibition may yield fruitful outcome in preventing resistance development in multiple cancer types. Activating *EGFR* mutations are an important oncogenic driver in a subset of lung adenocarcinoma, and *EGFR-TKIs* are effective in treating tumors with *EGFR* mutations. It has been shown that *EGFR* inhibitor-resistant NSCLC cells exhibit both increased expression and nuclear enrichment of *YAP* (Lee et al., 2016). In addition, *YAP* overexpression decreases *EGFR-TKI* sensitivity. Similarly, another study proposed *TAZ* as a mediator of intrinsic *EGFR-TKI* resistance in lung adenocarcinoma with *EGFR* T790M mutation. Monoclonal antibody cetuximab is

another important *EGFR* targeting modality, and *YAP* activation signature predicts fast tumor progression and poor disease control in colorectal cancer patients treated with cetuximab (Xu et al., 2015; Lee et al., 2015). Moreover, in *HER2*-positive breast cancer, high *TAZ* expression levels were associated with poor therapeutic response to *HER2* monoclonal antibody, trastuzumab, as well as chemotherapy combinations. Concordantly, a study also demonstrated that the anti-proliferative effect of *HER2* inhibitor lapatinib is influenced by *YAP* activity in vitro and in vivo. Reduction of *YAP* activity suppresses the growth of *HER2*-positive tumors, and a trend of increasing sensitivity to lapatinib is observed as *YAP* activity decrease. Knockdown of *YAP* has been also shown to sensitize cancer cells to *EGFR-TKI* erlotinib as well as to a small-molecule antagonist of survivin (Huang et al., 2013; Vici et al., 2014; Lin et al., 2015).

2.4.8 Crosstalk Hippo Pathway with other Signaling Pathways

Extensive crosstalk between the Hippo pathway and other signaling pathways makes up extremely complex cellular signaling networks that collectively affect cell proliferation and apoptosis (Figure 10).

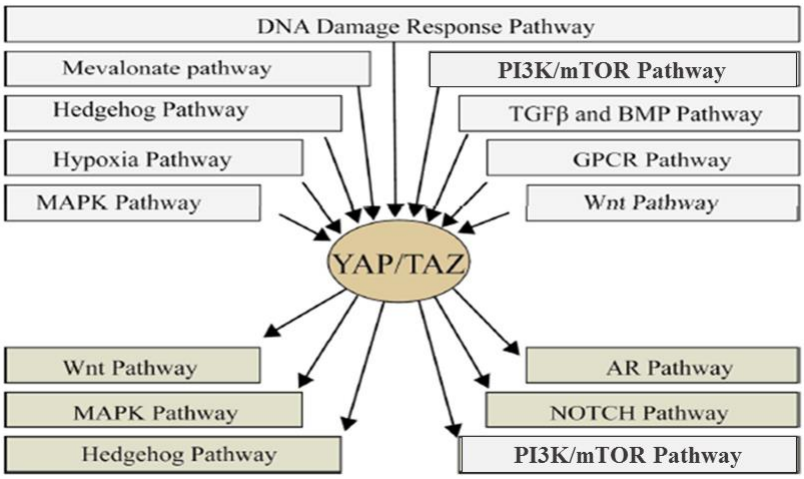


Figure- 10 Crosstalk Hippo pathway with other signaling pathways

2.4.8.1 Crosstalk Hippo Pathway with PIK3CA

PIK3CA, a catalytic subunit of *PI3K*, is one of the most frequently mutated genes identified in breast cancers (Nik-Zainal et al., 2016). One of the most common and best-characterized mutations of *PIK3CA* identified in breast cancer is H1047R in the kinase domain which causes increased catalytic activity. *PIK3CB*, another major catalytic subunit of *PI3K*, is also aberrantly activated in breast cancer cells; however, this is more commonly found to occur secondary to overexpression and/or gene amplification (Thorpe et al., 2014). Regardless of the mechanism, both the *PIK3CA-H1047R* mutation and *PIK3CB* overexpression result in hyperactivation of *PI3K*, enabling further exertion of its oncogenic functions (e.g., cell proliferation, antiapoptosis, and angiogenesis) through the *PI3K–PDK1–AKT* (*PI3K-AKT*) signaling pathway (Martini et al., 2014). In this pathway, the activation of *PI3K* can phosphorylate PI (Thorpe et al., 2014; Martini et al., 2014) P2 into PI (Thorpe et al., 2014; Martini et al., 2014; Vara et al., 2004) P3, which then interacts with *PDK1* and *AKT*, recruiting them to the inner leaflet of the cell membrane, where *PDK1* phosphorylates and activates *AKT*. Activated *AKT* subsequently phosphorylates a variety of downstream genes to cause increased cell proliferation, diminished apoptosis, and other oncogenic functions (Martini et al., 2014; Vara et al., 2004). Moreover, the activation of the *PI3K–AKT* pathway is frequently correlated with resistance to various anticancer therapies (Berns et al., 2007). Given this, many studies have been carried out to develop agents inhibiting *PI3K-AKT* for cancer treatment (Akinleye et al., 2013). Unfortunately, the existing drugs targeting the *PI3K–AKT* pathway often result in the development of drug resistance (Leroy et al., 2014), through unknown mechanisms. Recent studies have found that *PIK3CA* can positively regulate *YAP* in response to mitogen signals such as *EGF* treatment (Fan et al., 2013). In the studies on breast cancer showed that there is a systematic gain-of-function screening of kinases involved in mammary tumorigenesis. *PIK3CB* was identified as a kinase of interest and determined that *PIK3CB* positively regulates *YAP* and *TAZ* to promote mammary tumorigenesis both in vitro and in vivo. Similarly, *PIK3CA-H1047R* was found to positively regulate *YAP* and *TAZ* in mammary tumorigenesis, and also *YAP/TAZ* implicates in *PI3K*-related breast cancer and provides a new rationale for targeting *YAP/TAZ* for breast cancer treatment (Zhao et al., 2018). Studies reveal a functional link between Hippo and

PI3K-mTOR, providing a molecular basis for the coordination of these two pathways in organ size regulation. Hippo and TOR are two major signaling pathways involved in the regulation of organ size in Drosophila and mammals. Also, studies show that PI3K-mTOR is a pathway modulated by *YAP* to regulate cell size, tissue growth, and hyperplasia, and functional link between Hippo and PI3K-mTOR, providing a molecular basis for the coordination of these two pathways in organ size regulation (Tumaneng et al., 2012). TOR is well established as a central pathway regulating cell growth (cell size) by integrating intracellular and extracellular signals to stimulate protein translation (Kim et al., 2002). Because cell growth is required for proliferation, the function of the Hippo pathway is expected to be coordinated with TOR. Indeed, recent studies have provided evidence for a crosstalk between the Hippo and TOR pathways in Drosophila (Strassburger et al., 2012; Ye et al., 2012).

2.4.8.2 Crosstalk Hippo Pathway with *EGFR*

Similarly, mutations in the epidermal growth factor receptor *(EGFR)* and downstream effectors including the PI3K/AKT and mitogen-activated protein kinase *(*MAPK*)* signaling pathways are prominently featured among the driver mutations in a variety of human cancers (Stratton et al., 2011). Deregulation of *EGFR* function contributes to the growth and survival of cancer cells. Consequently, antibodies and small molecules that target *EGFR* and its downstream effectors have become important cancer therapeutics (Ciardiello et al., 2008). amphiregulin *(AREG)*, an epidermal growth factor receptor *(EGFR)* ligand, identified as a target of *TAZ*, that *AREG* functions in a non-cell-autonomous manner to mediate *EGF*-independent growth and malignant behavior of mammary epithelial cells. In addition, ablation of *TEAD* binding completely abolishes the *TAZ*-induced phenotype. Recent studies have revealed that breast cancer patient samples reveal a positive correlation between *TAZ* and *AREG* in vivo. In summary, *TAZ*-dependent secretion of *AREG* indicates that activation of the *EGFR* signaling is an important non-cell-autonomous effector of the Hippo pathway, and *TAZ*, as well as its targets, may play significant roles in breast tumorigenesis and metastasis (Yang et al., 2012).

2.4.8.3 Crosstalk Hippo Pathway with P53

p53 is a pivotal tumor suppressor and a major barrier against cancer. Level of *p53* was directly correlated with Hippo pathway activity in tetraploid cells. When the Hippo pathway was active, the amount of *p53* increased in the cell; when the Hippo pathway was inactive, p53 levels were low. *LATS2*, a core Hippo pathway kinase, is a negative regulator of *p53*. When the Hippo pathway is active, *LATS2* binds to *MDM2*, an E3 ubiquitin ligase, and prevents it from interacting with and degrading *p53*. Thus, an activated Hippo pathway stabilizes and increases *p53* levels in the cell, decreasing proliferation two-fold in tetraploid cells. When the Hippo pathway is inactive, *MDM2* is free to interact with *p53*, and repress its tumor suppressor functions (Ganem et al., 2007; Aylon et al., 2006). Silencing of the Hippo pathway tumor suppressors *LATS1* and *LATS2* in nontransformed mammary epithelial cells reduces *p53* phosphorylation and increases its association with the *p52 NF-κB* subunit. Moreover, it partly shifts *p53*'s conformation and transcriptional output toward a state resembling cancer-associated *p53* mutants and endows *p53* with the ability to promote cell migration. Notably, *LATS1* and *LATS2* are frequently down-regulated in breast cancer and reported that compromised *LATS* expression, seen in many tumors, alters wild-type *p53* to induce migration, and *LATS* knockdown reduces *p53* phosphorylation and changes *p53*'s protein interactome, increasing its binding to the *NF-κB p52* subunit. In addition, it partially alters *p53*'s conformation and favors a *p53* transcriptional program reminiscent of cancer-associated *p53* mutants. Hence, by reducing *LATS* expression, tumors that retain wild-type *p53* may convert it from a tumor suppressor to a tumor facilitator(Noa et al., 2015).

2.4.8.4 Crosstalk Hippo Pathway with Wnt

The Hippo pathway regulates Wnt/β- Catenin pathway through multiple mechanisms. *YAP* and β-catenin are recruited to *Sox2* and *Snai2* genes through *TEAD* and *TCF* transcription factors, respectively, and β-catenin forms a ternary complex with *YAP* and the transcription factor *TBX5*. Low of Hippo pathway activity cause to enhanced *TAZ* and decrease *TAZ-DVL* binding in the cytoplasm, which results in enhanced *CK1*-mediated phosphorylation of *DVL*, accumulation of β-Catenin in nuclear and induce of *Wnt*-target genes (Heallen et al., 2011; Rosenbluh et al., 2012; Varelas et

al., 2010). Cytoplasmic *YAP* restricts high *Wnt* signaling partly by limiting the activity of *DVL* and phosphorylated *YAP/TAZ* suppress *Wnt* signaling by directly binding to β-catenin and retaining it in the cytoplasm, and dephosphorylated *YAP/TAZ* promotes nuclear translocalization of *SHP2*, which in turn stimulates *TCF/LEF*- and *TEAD*-target genes through promoting tyrosine dephosphorylation of parafibromin (Barry et al., 2013; Imajo et al., 2012; Tsutsumi et al., 2013), and in the depletion of *Wnt* pathway, *GSK3*-phosphorylated β-catenin serves as a critical scaffold for *TAZ* recognition by the *β-TrCP* E3 ubiquitin ligase, suggesting that *Wnt* signaling regulates *TAZ* in a way that depends on the β-catenin destruction complex. Studies demonstrated a novel function of *APC* as a scaffold protein that *YAP/TAZ* phosphorylated interacting with *Sav1* and *LATS1*, and the tribbles homolog 2 (*TRIB2*), a direct target of Wnt/TCF, promotes protein stabilization of the *YAP* through interaction with the *β-TrCP* ubiquitin ligase. Interestingly, a recent study showed that β Catenin/TCF4 complexes directly regulate *YAP* gene expression through binding a DNA enhancer element within the *YAP* gene. Taken together, these data suggest a close integration between the Hippo and Wnt/β-Catenin pathways (Figure 11) (Azzolin et al., 2012; Cai et al., 2015; Konsavage et al., 2012; Wang et al., 2013).

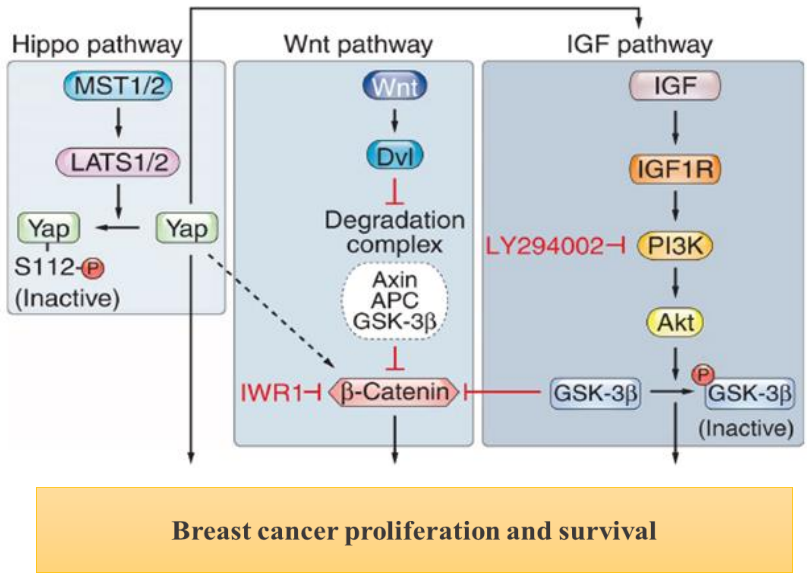


Figure. 11 The Hippo signaling pathway and antagonize the *WNT* signaling pathway (Konsavage et al., 2012)

2.4.8.5 Crosstalk Hippo Pathway with MAPK

Tumor formation often involves the inappropriate activation of regulatory pathways such as MAPK, that play vital roles in controlling growth and cell fate decisions. *EGFR* and Hippo signaling create a positive feedback loop that *EGFR/Ras* pathway stabilizes *YAP* through downregulation of the ubiquitin ligase complex substrate recognition factors SOCS5/6. *YAP* expression can be up-regulated through *EGFR* activation (Hong et al., 2014; Urtasun et al., 2011) and *YAP* induces the expression of epidermal growth factor receptors (*EGFR*, *ERBB3*) and production of *EGF*-like ligands (*HBEGF, NRG1, NRG2,* and *AREG*), which, in turn, activates *YAP* and stimulates cancer cell growth (He et al., 2015). Expression of activated forms of *RAF* or *MEK* increases *YAP* levels and reduces *YAP* phosphorylation through promoting phosphorylation of the Ajuba family protein *WTIP* binding to *LATS35* (Reddy et al., 2013) and also *YAP* rescues cell death in *KRAS* dependent cells upon suppression of *KRAS* and is required for *KRAS*-induced cell transformation and oncogenic *RAS* induces post-transcriptional modification of *YAP* through the MAPK pathway and augments its transcriptional activity (Li et al., 2014; You et al., 2015; Zhang et al., 2014). MAP4K4 interacts with *LATS* and promotes inhibition of *YAP*. On the other hand, several studies have been reported that *YAP* acts upstream of *ERK1/2* to promote cell survival, migration, and invasion in cancer cells. *JNK1/2* as kinases that robustly phosphorylate *YAP* and regulate its function in apoptosis (Danovi et al., 2008; Xu et al., 2011; Wang et al., 2014). Downregulation of c-Jun using siRNA resulted in reduced levels of endogenous *YAP* and *JNK* promotes binding between *LIMD1* and *LATS1* through direct phosphorylation of *LIMD1*, in turn, inhibits *YAP*. *YAP* negatively controls phosphorylation of MAPK14/p38 at *Thr180/Tyr182* (p-p38) through inhibition of *BTRC* expression. Taken together, MAPK and Hippo signaling regulate each other and form a positive feedback loop in human cancers (Codelia et al., 2014; Wang et al., 2016; Sun et al., 2013).

3. MATERIAL and METHODS

3.1. Materials

3.1.1. Used Equipment

- Refrigerated Centrifuges (Beckman Coulter, ABD)
- Refrigerated Centrifuges (Labogene, Denmark)
- Vortex (VELP Scientifica, İtaly)
- Shaker Incubator (JSR, Korea)
- Thermo-Shaker (Biosan, Latvia)
- -80°C freezer (Panasonic, Japan)
- -80°C freezer (Nuve, Turkey)
- -20°C freezer (Bosch, Turkey)
- -20°C freezer (Ugur, Turkey)
- Ice Systems (Scotsman, USA)
- Ice Systems (Samsung, Turkey)
- UV-Vis Spektrofotometre/Nano Drop (Beckman Coulter, USA)
- Thermal Cycler (Bio-Rad, California, USA)

- Profilex PCR system (Thermo Fisher Scientific, USA)
- UV Imaging Device (Vilber Lourmat, Germany)
- StepOnePlus™ System Real-Time- PCR (Applied Biosystem, USA)

3.1.2. Used Materials

- Agarose (Lonza, Switzerland)
- Ethidium bromide (Amresco, USA)
- Ethanol (Sigma Aldrich, USA)
- FFPE RNA Kit, 50 preps Omega / R6954-01 (Omega, Germany)
- High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA)
- TaqMan® Gene Expression *YAP* Assay (Thermo Fisher Scientific, USA)
- TaqMan® Gene Expression *TAZ* Assay (Thermo Fisher Scientific, USA)
- TaqMan® Gene Expression *LATS1/2* assay (Thermo Fisher Scientific, USA)
- TaqMan® Gene Expression *MST1/2* assay (Thermo Fisher Scientific, USA)
- TaqMan® Gene Expression *GAPDH* Assay (Thermo Fisher Scientific, USA)
- TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific, USA)

3.2. Methods

3.2.1. Clinical Samples and Ethics Statement

We investigated formalin-fixed, paraffin-embedded archival tumor and normal tissues from 100 patients with primary resected invasive ductal breast carcinoma, who underwent surgery during the years 01.01.2010- 01.09.2018 at the Bursa Uludag University (Turkey), and the supervision of sample selection by an

experienced pathologist. The usage of human archival tissue for molecular analysis was approved under the number (BUU 2018-3/25) by the local Ethics Committee.

3.2.2. RNA Extraction

3.2.2.1. Deparaffinization of FFPE Tissue

Archived Formalin-fixed, Paraffin-embedded (FFPE) tissue samples were used for profiling gene expression analysis. Two pieces of 10-µm thick sections FFPE tissue from each paraffin block were collected in sterile 1,5 mL eppendorf tubes and were baked at 56 °C for 15 min to soften the paraffin wax, followed by deparaffinization in xylene and 100% ethanol. In order to remove paraffin from FFPE tissue samples, two pieces of FFPE tissue sections were added with 1 mL of 100% xylene, heated for 5 min at 50 °C to melt the paraffin, and was then centrifuged for 2 min at room temperature at 16,000×g to per µL at the tissue. After centrifugation, xylene was removed and 1 mL of 100% ethanol was added to mix the sample which was again centrifuged at 16,000 ×g for 3 min at room temperature. After centrifugation, ethanol was discarded without disturbing the pellet. The ethanol washing process was repeated twice, during which residual ethanol was removed as much as possible without disturbing the pellet. Finally, the pellet was air-dried for approximately 25 min.

3.2.2.2. Total RNA Extraction

Total RNA was extracted from the tissues using OMEGA reagent (Omega, Germany) according to the manufacturer's instructions. The RNA was eluted in 50 µL of preheated Elution Solution and quantified on a UV-Vis Spektrofotometre/NanoDrop (Beckman Coulter, USA) (Table 1). The RNA samples were immediately stored at -80 C, until they were reverse transcribed into cDNA.

Table. 1 The 260/280 ratio and RNA concentration (ng/µl) measured on spektrofotometre for FFPE tumor and normal tissue samples.

NO Sample	Tumor tissue	The ratio of absorbance (260/280)	RNA concentration ng/µl	Normal tissue	The ratio of absorbance (260/280)	RNA concentration ng/µl
1	T	2	752	N	1,93	80
2	T	1,95	594	N	1,85	71
3	T	2	654	N	1,97	327
4	T	1,7	58	N	1,9	133
5	T	1,93	439	N	1,9	359
6	T	1.9	539	N	2	71
7	T	2	591	N	2	50
8	T	1,7	130	N	1,95	154
9	T	1,7	133	N	1,81	235
10	T	1,5	1000	N	1,94	205
11	T	1,3	1031	N	1,94	164
12	T	2	251	N	1,9	130
13	T	2	501	N	2	207
14	T	2	145	N	2,1	149
15	T	2	701	N	1,8	390
16	T	1,9	393	N	1,8	82
17	T	1,9	94	N	1,8	41
18	T	2	449	N	2	510
19	T	2	335	N	1,9	193
20	T	1,9	665	N	2	272
21	T	1,7	1017	N	1,9	137
22	T	1,6	973	N	2	216
23	T	1,6	1021	N	2	830
24	T	2	209	N	2	287
25	T	1,6	1028	N	2	336
26	T	1,9	574	N	2	95
27	T	1,7	970	N	1,8	843
28	T	2	147	N	1,9	465
29	T	1,9	91	N	1,9	147
30	T	2	207	N	1,4	343
31	T	1,54	378	N	2,1	454
32	T	2	77	N	2	767
33	T	1,6	58	N	1,4	29
34	T	2	852	N	2	50
35	T	1,8	977	N	2	541

36	T	2	224	N	2	148
37	T	1,9	805	N	2	544
38	T	1,7	1008	N	2	281
39	T	1,5	1034	N	1,8	52
40	T	1,8	998	N	2	273
41	T	1,8	941	N	2	182
42	T	1,7	1022	N	2	390
43	T	1,8	931	N	2	214
44	T	1,6	926	N	1,9	135
45	T	1,8	85	N	2	87
46	T	1,4	1014	N	2	712
47	T	1,9	588	N	1,9	128
48	T	1,3	1034	N	2	254
49	T	2	591	N	2,1	118
50	T	2	129	N	2	610
51	T	1,1	1002	N	2	156
52	T	1,3	1004	N	2	212
53	T	1,2	1015	N	2	321
54	T	2	399	N	1,9	45
55	T	1,5	991	N	2	136
56	T	1,6	963	N	1,1	1015
57	T	1,2	1041	N	2,1	88
58	T	1,1	1060	N	2	98
59	T	1,3	1060	N	2	444
60	T	2	924	N	2,1	721
61	T	1,9	737	N	1.9	465
62	T	1,3	1011	N	1,6	698
63	T	1,9	291	N	1,7	46
64	T	2	777	N	1,6	396
65	T	1,7	993	N	1,9	126
66	T	1,1	1052	N	2	94
67	T	1,9	882	N	1,7	63
68	T	1,4	1000	N	1,9	371
69	T	1,1	1061	N	2	185
70	T	1,2	1061	N	1,9	65
71	T	2	514	N	1,8	128
72	T	2	514	N	2	123
73	T	1,7	977	N	2,1	71
74	T	1,9	87	N	2	353
75	T	2,1	292	N	2,1	31
76	T	2,1	609	N	2,1	156
77	T	2,1	581	N	2,1	509
78	T	2	107	N	2	70
79	T	2,1	345	N	2,1	87
80	T	1,6	254	N	2,1	126
81	T	2,1	445	N	1,3	98

82	T	2	466	N	2	638
83	T	1,9	898	N	2	394
84	T	2	359	N	2	316
85	T	1,83	894	N	1,9	80
86	T	1,7	236	N	2	95
87	T	2	702	N	1,5	254
88	T	2	200	N	2,1	250
89	T	1,9	360	N	2	420
90	T	2	424	N	2	525
91	T	2	26	N	2	671
92	T	2,1	202	N	1,6	30
93	T	1,64	202	N	1,53	496
94	T	2	415	N	1,9	155
95	T	2	366	N	2	144
96	T	2	453	N	1,9	93
97	T	2,1	634	N	2,1	104
98	T	2,1	5.4	N	2	66
99	T	1,8	351	N	1,9	184
100	T	1,8	541	N	2	541

3.2.3. cDNA Synthesis

Reverse transcription was performed by the TaqMan High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, USA) after RNA extraction. The 20 µL reverse transcription reaction contained dNTPs, MultiScribe Reverse Transcriptase (50 U/ µL), 10x Reverse Transcription Buffer, Random Primer, nuclease-free water, and 10 µL RNA (Table 2).

Table. 2 Preparing the 2x Reverse Transcription Master Mix

Component	**Volume/Reaction (µL)**
10✕ RT Buffer	2.0
25✕ dNTP Mix (100 mM)	0.8
10✕ RT Random Primers	2.0
MultiScribe™ Reverse Transcriptase	1.0
Nuclease-free H2O	4.2
Total per Reaction	10.0

Set the reaction volume to 20 µL.

The reaction was carried out at 4 steps (Table 3) on (Bio-Rad, California, USA). The cDNA samples were stored at -20 °C until further usage.

Table. 3 Performing Reverse Transcription

	Step 1	Step 2	Step 3	Step 4
Temperature (°C)	25	37	85	4
Time	10 min	120 min	5 min	∞

3.2.4. TaqMan® Gene Expression and Probes

All probes (Applied Biosystems, Foster City, CA, USA) in this study, based on the mRNA sequences of target *YAP*, *TAZ*, *LATS1/2*, *MST1/2* and reference gene *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase) which were obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Table 4).

Table. 4 TaqMan® Gene Expression Assays to evaluate the yield of cDNA conversion.

Gene Target	**Kit**	**Assay ID**	**Amplicon Length (bp)**
GAPDH	TaqMan® Gene Expression Assays, *GAPDH* [Human]	Hs03929097_g1	58
YAP	TaqMan® Gene Expression Assays, *YAP* [Human]	Hs00902712_m1	65
TAZ	TaqMan® Gene Expression Assays, *TAZ* [Human]	Hs00902887_sH	87
LATS1	TaqMan® Gene Expression Assays, *LATS1* [Human]	Hs 01125524_m1	80
LATS2	TaqMan® Gene Expression Assays, *LATS2* [Human]	Hs 01059009_m1	80
MST1	TaqMan® Gene Expression Assays, *MST1* [Human]	Hs00360684_m1	98
MST2	TaqMan® Gene Expression Assays, *MST2* [Human]	Hs00169491_m1	98

3.2.5. Real-Time *qRT-PCR*

The RT step synthesizes a cDNA copy of the RNA template. After denaturation, primers and probe anneal to their targets. The probe contains a reporter dye at the 5' end and a quencher (Q) at its 3' end. During the polymerization step, the 5' nuclease activity of the Taq polymerase displaces and cleaves the probe. This physically separates the reporter dye and quencher dyes, resulting in reporter fluorescence. The increase in signal is directly proportional to the number of molecules released during that cycle. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. (Figure 12).

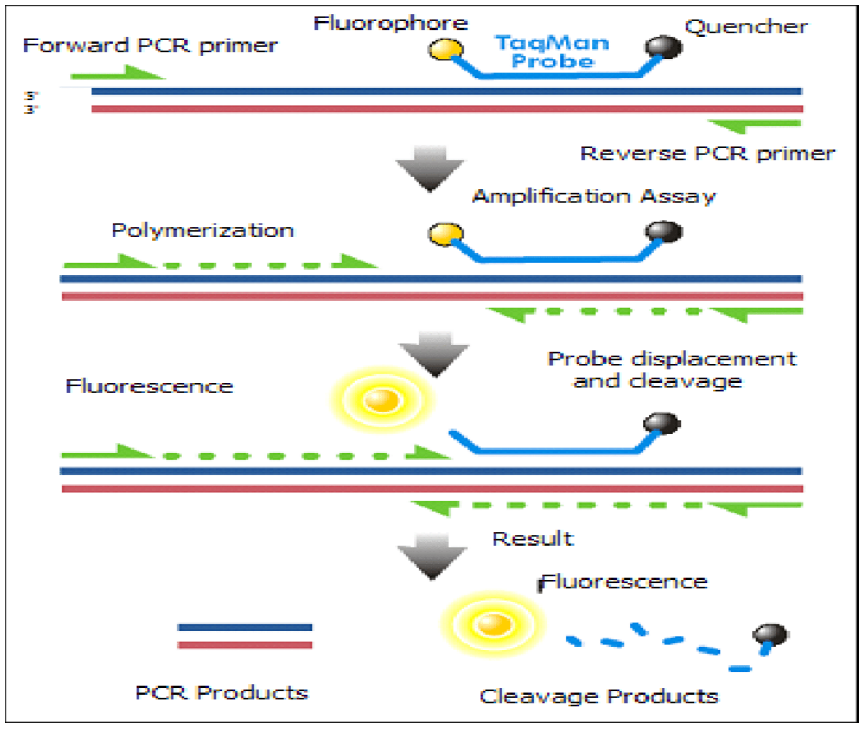


Figure. 12 TaqMan® Gene Expression Assays (Raymaekers et al., 2009)

Cleavage of the probe:

Separates the reporter dye from the quencher dye, increasing the reporter dye signal. Removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Thus, inclusion of the probe does not inhibit the overall PCR process. Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced. (Figure 12) (Raymaekers et al., 2009).

3.2.6. Standard Curve Construction and Amplification Efficiency Optimization

Quantification of RNA with real-time PCR can be performed by the standard curve and the comparative method. The first method is based on the close relationship between the input copy number and the increase of fluorescence in the exponential phase. Quantification can be either absolute or relative. Absolute quantification requires the construction of a standard curve, plotting the Ct values against the logarithm of the initial copy numbers of standards with known concentration. Standard material must be stable, reliable, and precisely quantified. The copy numbers can be calculated after linear regression of the standard curve. Absolute quantification allows the exact determination of copy number per cell, per total RNA concentration or per sample matrix. Relative quantification determines the changes of steady-state transcription of a gene. A relative standard curve consists of a dilution series created with a calibrator with arbitrary units. To circumvent the use of standard material and standard curves, relative changes in the expression of the target gene can also be determined by the use of the comparative ΔΔCT, when PCR efficiencies are the same, or by the mathematical model proposed by Pfaffl, when PCR efficiencies are different. To compensate for differences in the amount of biological material in the tested sample, normalization is necessary. Many normalization procedures have been suggested but the most popular strategy is normalization to internal reference genes. Finding appropriate reference genes for data normalization is a problem because evidence suggests that there is no universal reference gene with a constant expression in all tissues (Raymaekers et al., 2009).

In the present study, the reaction mix for each sample using the components listed in Table 5. For optimal performance of TaqMan, ®Gene Expression used 100 ng of cDNA. Then the appropriate volume of each reaction mixture transferred to each well of an optical plate, and the plate covered with a MicroAmp® Optical Adhesive Film. For standard 96- well plates and centrifuged the plate briefly to spin down the contents and eliminate air bubbles from the solutions. The plate placed in the Applied Biosystems real-time PCR instrument (Applied Biosystems, Inc.). The

assays were started by denaturation for 2 min at 50 °C, 10 min at 95 °C and followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min (Table 6).

Table. 5 Reaction mix for each sample

Component	**Volume (µL) per reaction (20-µL rxns.)**	**Final Conc.**
TaqMan® Universal Master Mix II (2✕)	10,0	1X
TaqMan® Gene Expression Assay (20✕)	1,0	1X
cDNA template + H2O	9,0	100 ng
Total Volume	**20,0**	-

Table. 6 Thermal Cycling Parameters

System	incubation	Polymerase activation	PCR	
	Hold	Hold	Cycle (40 cycles)	
			Denature	Anneal/extend
Temp. (°C)	50	95	95	60
Time (min:sec)	2,0 min	10,00 min	00,15 sec	1,00 min

RT-PCR data analysis: The experimental data were processed by $2^{-\Delta\Delta CT}$ on the premise that the amplification efficiency of the target genes (*YAP1, TAZ, LATS1/2, MST1/2*) and reference gene. The average CT value from samples and ΔCt value ($\Delta Ct = Ct_{Target\ gene} - Ct_{GAPDH}$) was calculated, and $2^{-\Delta\Delta CT}$ ($CT = CT_{tumor\ sample} - CT_{control\ sample}$) was computed.

YAP1, TAZ, LATS1/2, MST1/2 cutoff values were predefined based on previous studies. Since different platforms, normalization strategies and primer/probe lots had been used a constant target-specific shift in Ct values between previous and current

assay conditions occurred. The cutoffs from the published previous studies were therefore transformed by the addition of an offset.

3.2.7. Data Analysis and Statistics

Statistical analysis was performed using SPSS version 19.0 (IBM, Armonk, NY, USA).

Survival analysis was performed using Kaplan-Meier estimates. All tests were 2-sided, and the significance level was set at 0,05. Also, graphs made in GraphPad program, and p- values used from SPSS.

4. RESULTS

4.1 Patient Characteristics

Patient ages ranged from 29 to 73 (median: 52, mean: 53.19). All of the patients, classified as IDC breast cancer. The baseline characteristics of the study group are presented in Table 7. Baseline regimens of treatments are shown in Table 8. A total of 100 invasive ductal carcinoma breast cancer were in this study. Hormone receptor and *HER2* status were obtained from pathology reports. Follow-up means in this study was mean 60 months, and until the follow-up was July 10, 2019.

Table. 7 Baseline Clinical and Pathologic Characteristics of the Invasive Ductal Carcinoma Breast Cancer Patients (n=100)

Characteristics	Number	Number (%)
Age		
< 50	40	%42.55
≥ 50	54	%57.45
Tumor localization		
Right	33	%35.48
Left	41	%44.09
Bilateral	19	%20.43
Grade		
I/ II	50	%54.35
III	42	%45.65
Lymph node		
N0: node-negative	48	%51.07
N1: metastasis involving 1–3 nodes	27	%28.72
N2: at least 4 nodes	19	%20.21
Tumor size (cm)		
< 3 cm	57	%64.04
≥ 3 cm	32	%35.96
Ki-67		
≤15%	29	%32.22
15.01%–35%	40	%44.45
>35%	21	%23.33

Lymphatic invasion		
Positive	24	%26.67
Negative	66	%73.33
Perineural invasion		
Positive	18	%19.78
Negative	73	%80.22
***ER* status**		
Positive	71	%77.17
Negative	21	%22.83
***PR* status**		
Positive	58	%63.04
Negative	34	%36.96
***HER2* status**		
Positive	36	%39.13
Negative	56	%60.87
Subtypes		
Luminal A	46	%52.27
Luminal B	25	%28.41
TNBC	6	%6.82
HER2-enriched	11	%12.50
Metastasis/ Recurrence		
With Recurrence	35	%50.72
Without Recurrence	34	%49.28
In situ component		
No-DCIS (0)	10	%14.70
L-DCIS (<25%)	30	%44.12
H-DCIS (≥25%)	28	%41.18

Table. 8 Baseline patient characteristics and regimens of treatments (n =100)

Characteristics	Number	Number (%)
Chemotherapy		
Neoadjuvant treatment	32	%41.56
Adjuvant treatment	45	%58.44
Radiotherapy		
Yes	72	%73.47
No	26	%26.53
Hormonotherapy		
Yes	53	%55.79
No	42	%44.21

4.2 Expression of *YAP1, TAZ, LATS1, LATS2, MST1* and *MST2* mRNA in IDC breast cancer

Housekeeping genes were widely used to detect the expression of *YAP1*, *TAZ*, *LATS1*, *LATS2*, *MST1*, and *MST2* genes. The relative gene expression of

individual tumor tissue of each patient was determined by using the $2^{-\Delta\Delta CT}$ comparative methods and calculated by Sabiosciences' data analysis software (https://dataanalysis.qiagen.com).

Comparative RNA expression analysis in tumor versus normal cells (calibrator) was performed as follows:

1. Tumor tissue: $\Delta CT = CT_{(Target)} - CT_{(GAPDH)}$
2. Normal tissue: $\Delta CT = CT_{(Ttarget)} - CT_{(GAPDH)}$
3. $\Delta\Delta CT = \Delta CT_{(Tumor\ tissue)} - \Delta CT_{(Normal\ tissue)}$
4. Ratio = $2^{-\Delta\Delta CT}$

According to the ROC analysis, the cut-off value of *YAP1, LATS1, LATS2, MST1, and MST2* was determined. *YAP1, LATS1, LATS2, MST1, and MST2* mRNA gene expression differences between tumors and normal tissues were compared (Table 9 and Figure 13).

Our study showed that *YAP1*, *LATS1*, and *LATS2* expressions in tumor tissues significance was down-regulated. No significant difference in *TAZ*, *MST1*, and *MST2* mRNA expressions was found between the normal and IDC tumor tissues.

Table. 9 Roc analysis; *YAP1*, *LATS1*, and *LATS2* mRNA gene expression differences between tumors and normal tissues

Assays	Sensitivity %	AUC	Specificity %	95% CI	p-value
YAP1	72,0%	0,739	73,0%	0,673- 0,799	***P= 0,000**
TAZ	96,9%	0,536	15,3%	0.4582-0.6021	P= 0, 459
LATS1	83,7%	0,640	43,0%	0,968- 0,707	***P= 0,0004**
LATS2	80,4%	0,695	55,4%	0,623- 0,761	***P= 0,0001**
MST1	83,1%	0,557	32,5%	0.4751-0.6372	P= 0,219
MST2	89,2%	0,505	18,1	0.4267-0.5836	P= 0,906

ROC, Receiver operating characteristic; CI, Confidence interval.

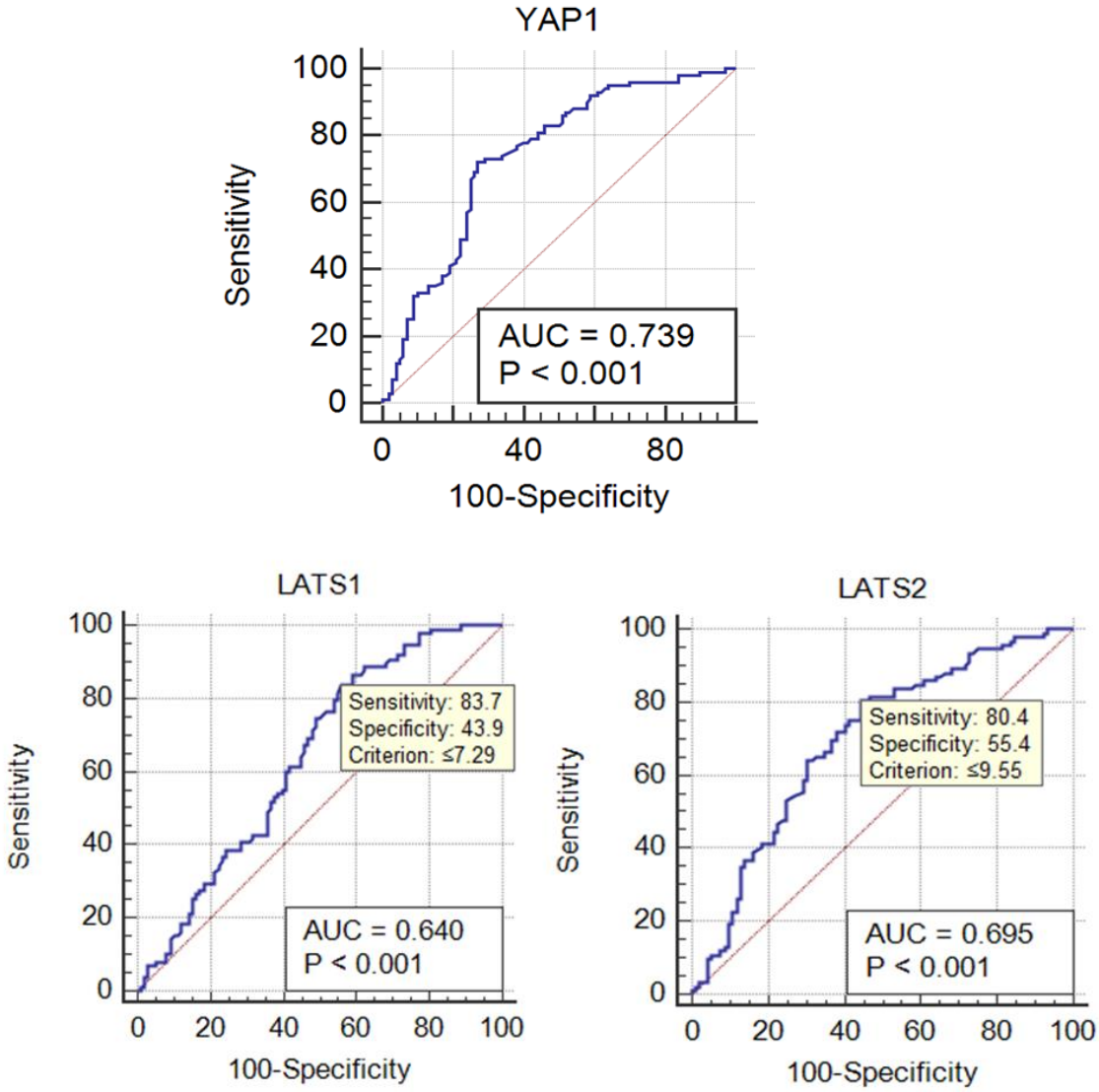


Figure. 13 ROC analysis, the cut-off value of *YAP1*, *LATS1*, and *LATS2* RT-PCR in tumor and normal tissue

The fold-change of *YAP1* expression in tumor tissues was approximately 0,44 down-regulated (*p*= 0,026), and the fold-change of *LATS1* and *LATS2* expressions in tumor tissues were 0,49 and 0,50 down-regulated, respectively (p= 0,001 and p= 0,000).

Fold change of *TAZ* was analyzed between the tumors and normal tissues, was 0,78 down-regulated (p= 0,630). Also, fold-change of *MST1* and *MST2* expressions in tumor tissues were 0,69 and 0,98 down-regulated, respectively (p= 0,224 and p= 0,818) (Table 10).

Graphs of differential expression (fold change) for *YAP1*, *TAZ*, *LATS1*, *LATS2*, *MST1*, and *MST2* mRNA gene expressions comparison between tumors and normal tissues were presented in Figure 14.

Table. 10 Gene expressions comparison between tumors and normal tissues.

PCR Array genes	Normal Tissue 2^(- Avg.(Delta(Ct))	Tumor Tissue 2^(- Avg.(Delta(Ct))	95% CI*	Fold Change	Fold Regulation (Up-Down Regulation)	p-value
YAP1	0,014076	0,006136	(0,27, 0,60)	0,4359	Down- Regulated	**0,026***
TAZ	0,003544	0,002791	(050, 1,08)	0,7874	Down- Regulated	0,630
LATS1	0,019132	0,011356	(0,17, 1,02)	0,4935	Down- Regulated	**0,001***
LATS2	0,001835	0,001104	(0,45, 1,21)	0,5019	Down- Regulated	**0,000***
MST1	0,007153	0,004929	(0,37, 1,01)	0,6891	Down- Regulated	0,224
MST2	0,01294	0,012773	(0,39, 1,58)	0,9871	Down- Regulated	0,818

The p values are calculated based on t-test of the replicate $2^{\wedge(-\text{Delta CT})}$ values for each gene in the control group and treatment groups.

* CI: Confidence interval

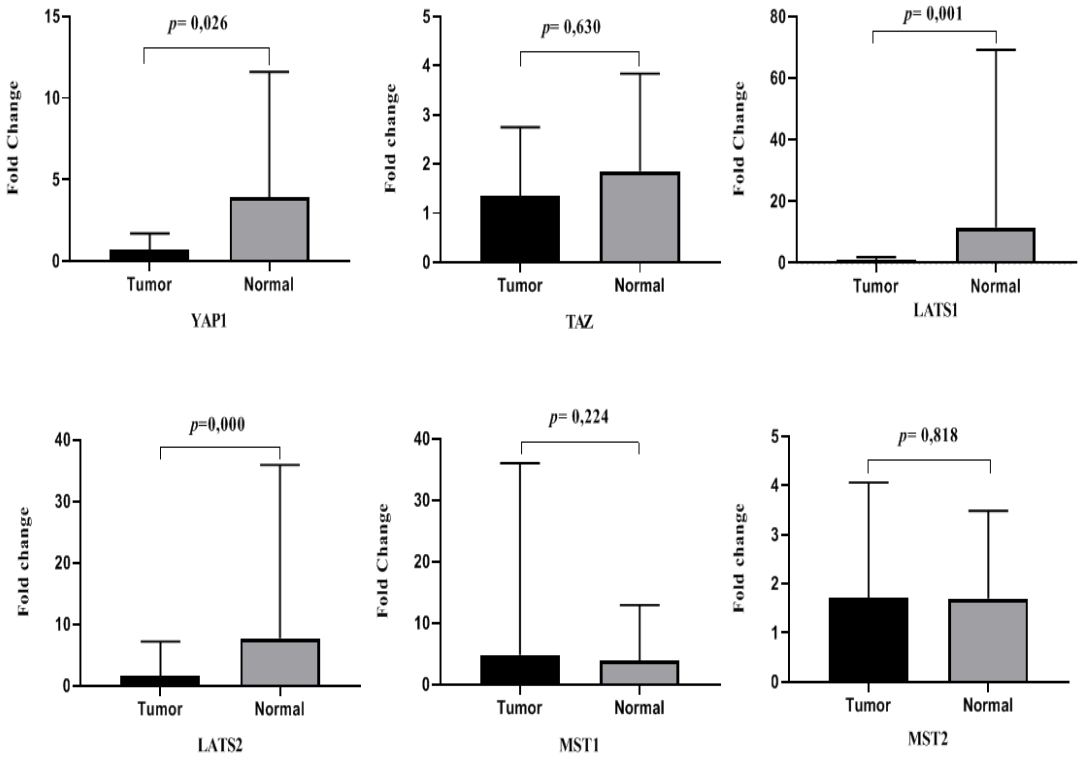


Figure. 14 *YAP1, TAZ, LATS1/2, MST1/2* expressions comparison between tumors and normal tissues.

4.3 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with Tumor Grade

We examined the potential association of *YAP1*, *LATS1*, and *LATS2* expressions abundance with low-grade and high-grade. The result indicated that a low level of *YAP1* with fold change 0.4371 was associated with histological grade (grade 1/2) of tumor tissues (*p*= 0,0352). The mRNA expression of *LATS1/2* no had a significant relation with tumor grade, but mRNA expressions of *LATS1/2* decreased in both grade3 and grade1/2 (Table 11, Figure 15).

Table. 11 Correlations between the expression levels of *YAP1 and LATS1/2* mRNAs in tumor tissues and the tumor grade

Gene	Normal Tissue	Tumor Tissue (Grade 3) n= 42				Normal Tissue	Tumor Tissue (Grade 1/2) n= 50			
	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.016129	0.00572	-2.8198	0.354	0.0883	0.028012	0.012244	-2.2979	0.437	**0.0352**
LATS1	0.030433	0.013556	-2.2449	0.445	0.5519	0.037213	0.01281	-2.9004	0.344	0.4846
LATS2	0.00239	0.000839	-2.8486	0.351	0.1468	0.002826	0.001716	-1.6465	0.607	0.7191

***p <0,05**

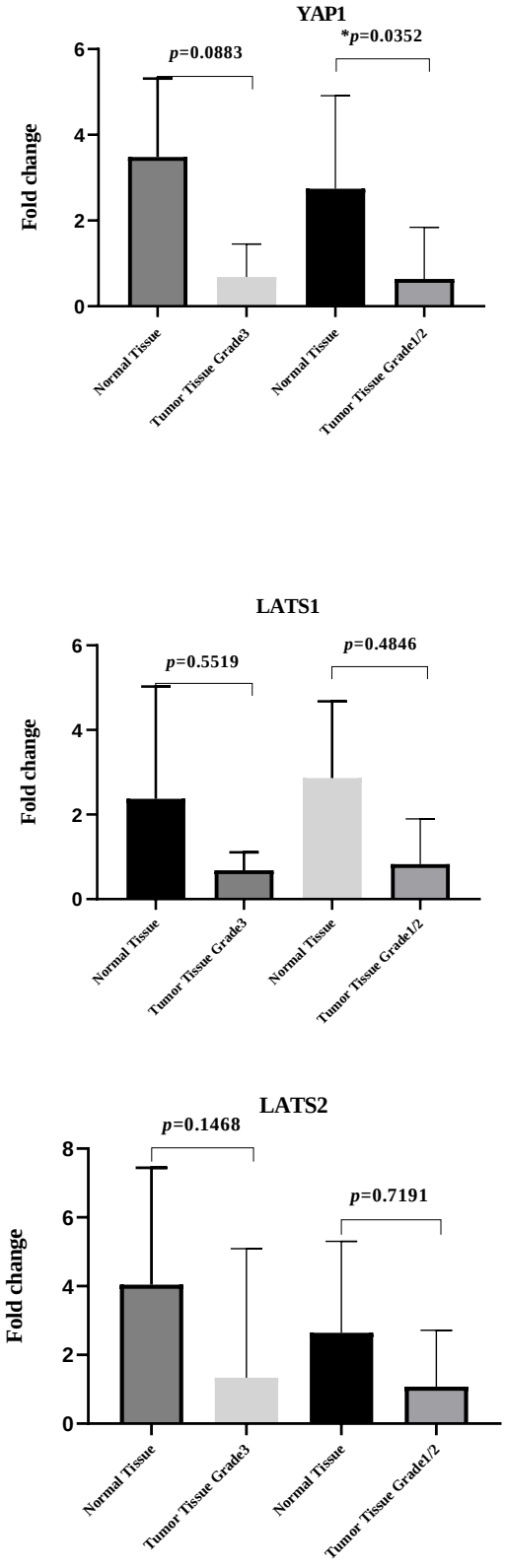


Figure. 15 *YAP1*, *LATS1*, and *LATS2* expressions correlation with tumor grade.

4.4 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with Tumor Size

We investigated the potential association of *YAP1*, *LATS1*, and *LATS2* expressions abundance with tumor size. The results indicated that downregulated of *YAP1* with fold change 0.3832 associated with tumor size (>3 cm) (*p*= 0,0276) (Table 12, Figure 16).

Table. 12 The Relationship between *YAP1, LATS1*and *LATS2* Expressions with Tumor Size

Gene	Normal Tissue	Tumor Tissue (Tumor Size (>3 cm)) n= 32				Normal Tissue	Tumor Tissue (Tumor Size (<3 cm)) n= 57			
	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.022021	0.008439	-2.6095	0.383	**0.0276**	0.021038	0.008301	-2.5343	0.394	0.1149
LATS1	0.025061	0.009707	-2.5818	0.387	0.1953	0.028409	0.013869	-2.0484	0.488	0.7858
LATS2	0.002661	0.001397	-1.9045	0.525	0.2589	0.003213	0.001571	-2.0447	0.489	0.1799

***p >0,05**

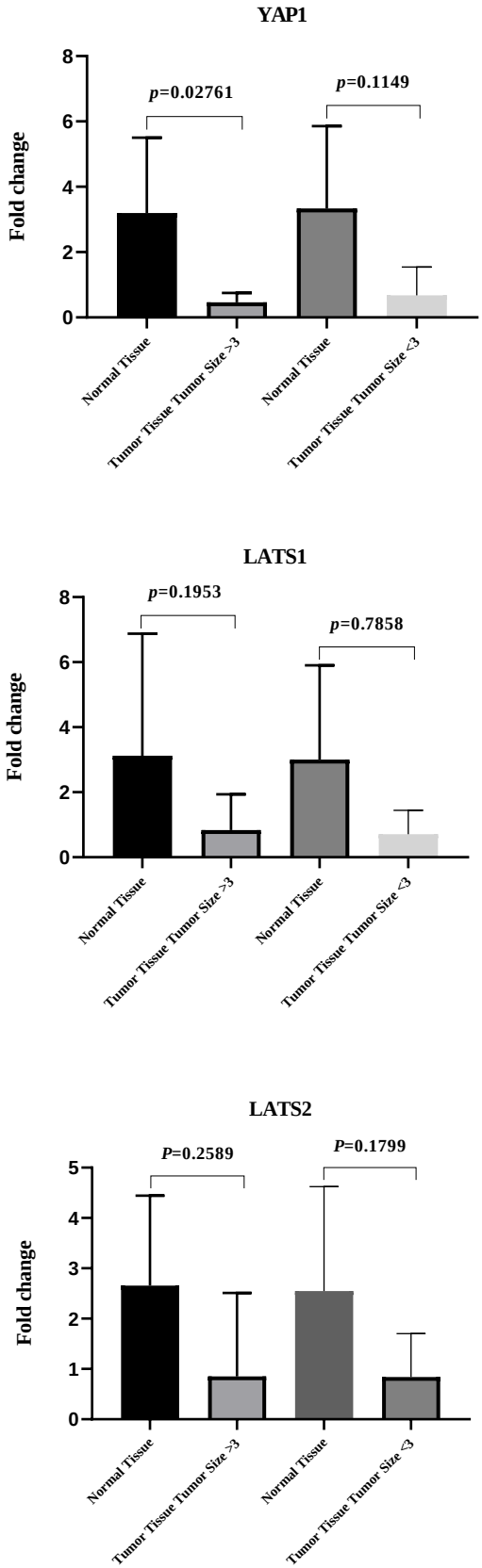


Figure. 16 he Relationship between *YAP1*, *LATS1*and *LATS2* Expressions with Tumor Size

4.5 The Relationship between *YAP1, LATS1*and *LATS2* Expressions with Recurrence/Metastasis

We examined the potential association of *YAP1*, *LATS1*, and *LATS2* expressions abundance with recurrence/metastasis. The results demonstrated that downregulated of *YAP1* with fold change 0.380 associated with metastasis-positive tumors (*p*= 0,0262). Also, the results showed that downregulated of *LATS1* with fold change 0,356 related with metastasis-positive tumors (*p*= 0,0178) (Table 13, Figure 17).

Table. 13 The Relationship between *YAP1, LATS1*and *LATS2* Expressions with Recurrence/Metastasis

Gene	Normal Tissue	Tumor Tissue (Recurrence/Metastasis (+)) n= 35				Normal Tissue	Tumor Tissue (Recurrence/Metastasis (-)) n= 34			
	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.017409	0.006617	-2.6312	0.380	**0.0262**	0.022335	0.008931	-2.5008	0.399	0.1141
LATS1	0.020099	0.009426	-2.1322	0.469	0.7509	0.032367	0.014485	-2.2346	0.447	0.7743
LATS2	0.002245	0.000799	-2.8089	0.356	**0.0178**	0.002777	0.001333	-2.0827	0.480	0.2124

***p <0,05**

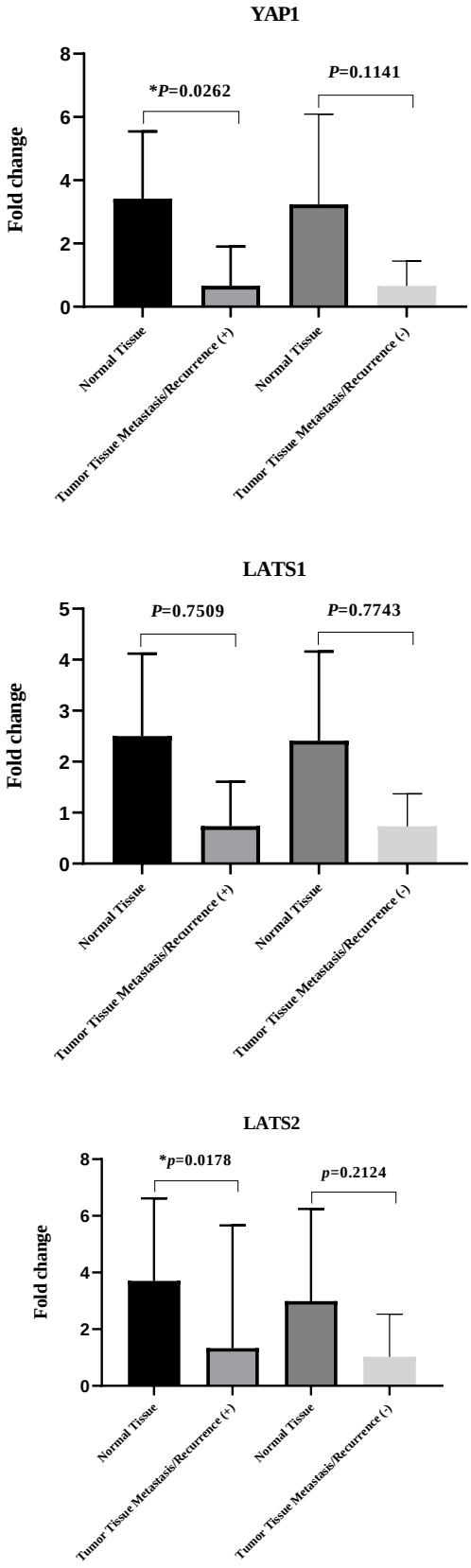


Figure. 17 The Relationship between *YAP1*, *LATS1*and *LATS2* Expressions with Recurrence/Metastasis

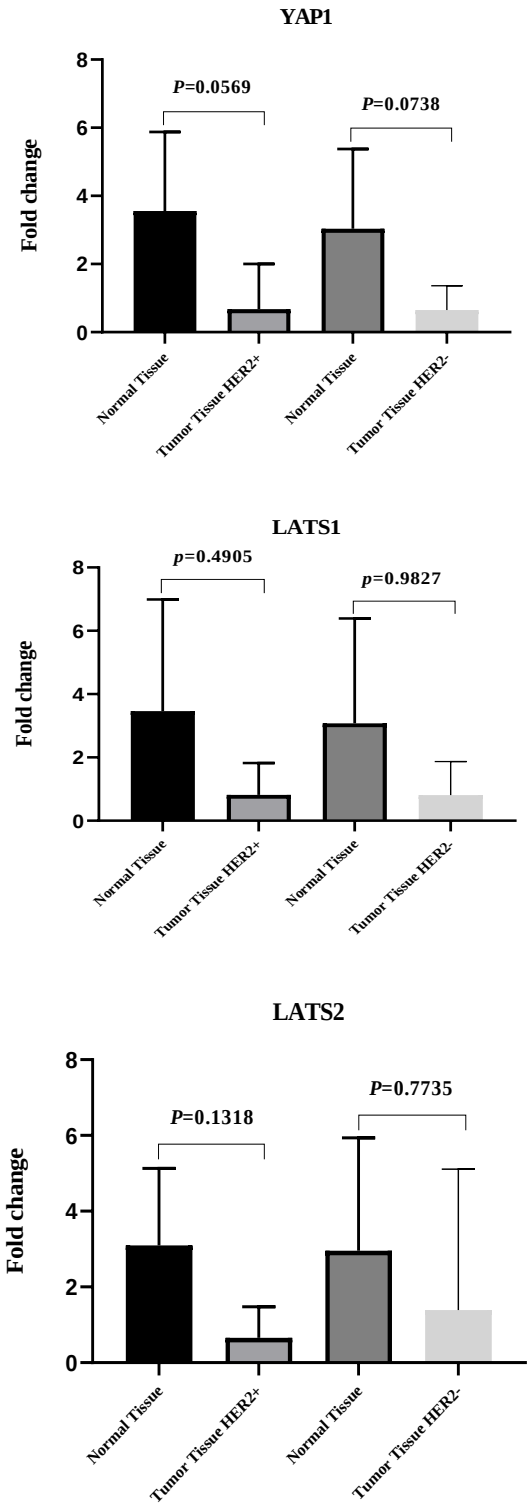
4.6 The Relationship between *YAP1, LATS1*and *LATS2* Expressions with *HER2* Status

We investigated the potential association of *YAP1*, *LATS1*, and *LATS2* expressions abundance with *HER2* status. The results demonstrated that downregulated of *YAP1* with fold change 0,356 associated with *HER2*-positive tumors; *p* value close to but not quite statistically significant (*p*= 0,0569) (Table 14, Figure 18).

Table. 14 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with *HER2* Status

Gene	Normal Tissue	Tumor Tissue (HER2 (+)) n= 36				Normal Tissue	Tumor Tissue (HER2 (-)) n= 56			
	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.015572	0.005544	-2.8089	0.356	0.0569	0.024444	0.009814	-2.4906	0.401	0.0738
LATS1	0.020333	0.00817	-2.4888	0.401	0.4905	0.49056	0.016306	-1.9529	0.512	0.9827
LATS2	0.003096	0.001256	-2.4648	0.405	0.1318	0.002603	0.001556	-1.6728	0.597	0.7735

***p <0,05**



Figur. 18 The Relationship between *YAP1*, *LATS1*and *LATS2* Expressions with *HER2* Status

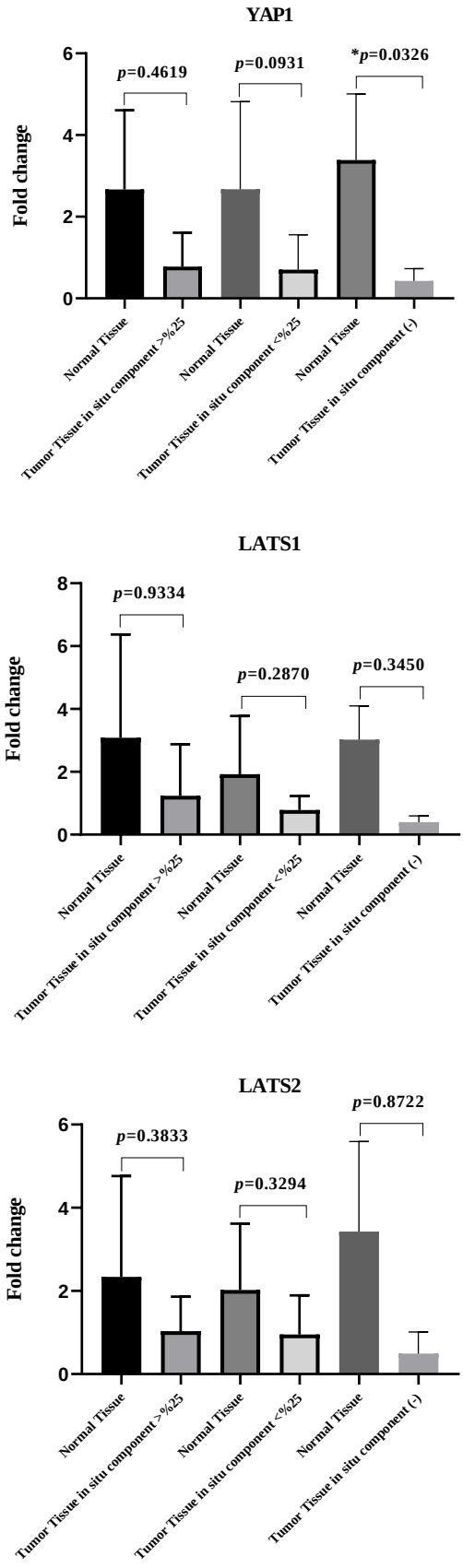
4.7 The Relationship between *YAP1, LATS1*and *LATS2* Expressions with in situ component

We examined the potential association of *YAP1, LATS1*, and *LATS2* expressions abundance with in situ component status. The results showed that downregulated of *YAP1* with fold change 0,3128 associated with in situ component-negative tumors (p= 0,0326) (Table 15, Figure 19).

Table. 15 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with in situ component

Gene	Normal Tissue	Tumor Tissue (in situ component ≥25%) n= 28				Normal Tissue	Tumor Tissue (in situ component <25%) n= 30				Normal Tissue	Tumor Tissue (in situ component (-)) n=10			
	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.022272	0.010314	-2.1595	0.463	0.4619	0.021803	0.010828	-2.0135	0.496	0.0931	0.022965	0.007183	-3.1969	0.312	**0.032**
LATS1	0.06458	0.03128	-2.0646	0.484	0.9334	0.021235	0.013245	-1.6032	0.623	0.2870	0.02361	0.009163	-2.5768	0.388	0.345
LATS2	0.002384	0.001582	-1.5069	0.663	0.3833	0.002756	0.001853	-1.4876	0.672	0.3294	0.002694	0.000808	-3.3334	0.3	0.872

***p >0,05**



Figur.19 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with in situ component

4.8 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with *PR* Status

We examined the potential association of *YAP1*, *LATS1*, and *LATS2* expressions abundance with *PR* status. The results demonstrated that downregulated of *YAP1* with fold change 0,4222 significant associated with *PR*-positive tumors (*p*= 0,0000) (Table 16, Figure 20).

Table. 16 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with *PR* Status

Gene	Normal Tissue	Tumor Tissue PR (+) n= 58				Normal Tissue	Tumor Tissue PR (-) n= 34			
	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.015022	0.006343	-2.3684	0.4222	**0.0000**	0.016109	0.005314	-3.0314	0.3299	0.0558
LATS1	0.022343	0.011613	-1.9239	0.5198	0.5550	0.036935	0.0142	-2.6011	0.3844	0.4936
LATS2	0.014557	0.011455	-1.2708	0.7869	0.4888	0.003424	0.001298	-2.6379	0.3791	0.1145

***p <0,05**

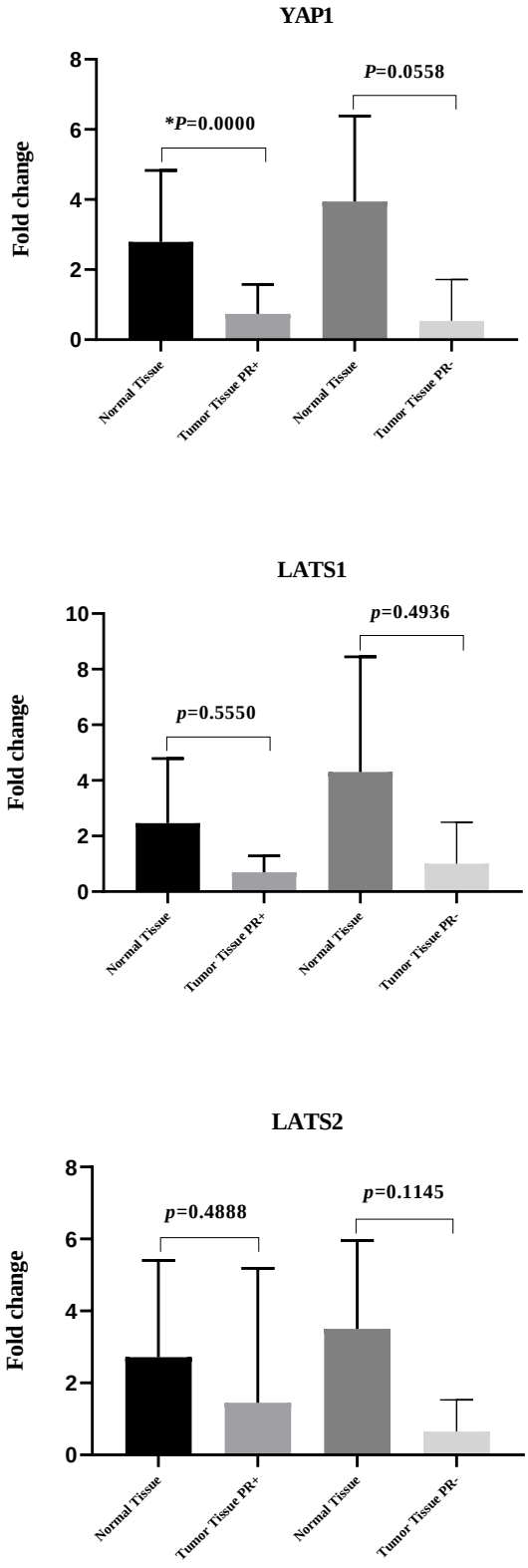


Figure. 20 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with *PR* Status

4.9 The Relationship between *YAP1, LATS1 and LATS2* Expressions with Ki-67 Proliferation Index

Cut points for Ki-67 and statistical evaluation Cut points for Ki67 were tested using the Cutoff Finder algorithm. Based on the results for the three endpoints pCR, DFS and OS as well as results from previous studies (Budczies J et al., 2012), we grouped Ki67 levels in three groups: low (≤15%), intermediate (15.01%–35%) and high (>35%). We evaluated the mRNA expression of *YAP1* and relation with Ki-67 proliferation index. Statistical analysis showed that levels of low-expression of *YAP1* were significantly correlated with Ki-67 proliferation index Ki-67>35% (*p*=0,0479). Also, Ki-67 15%–35% proliferation increased in tumors with low-level *LATS2* statistically was significant (p=0,0503) (Table 17, Figure 21).

Table. 17 Correlations between the expression levels of *YAP1 and LATS1/2*mRNAs in tumor tissues and the Ki-67 proliferation index.

Gene	Normal Tissue	Tumor Tissue (Ki-67 >35%) n= 21				Normal Tissue	Tumor Tissue (Ki-67 15%–35%) n= 40				Normal Tissue	Tumor Tissue (Ki-67 <15%) n= 29			
	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.015995	0.005443	-2.9388	0.3403	**0.0479**	0.021065	0.008508	-2.476	0.4039	0.1209	0.025566	0.011512	-2.2207	0.4503	0.2000
LATS1	0.019537	0.007914	-2.4688	0.4051	0.8573	0.032169	0.015558	-2.0676	0.4837	0.4138	0.033179	0.016913	-1.9618	0.5097	0.4914
LATS2	0.002583	0.001033	-2.5003	0.4	0.2502	0.002275	0.001107	-2.0546	0.4867	**0.0503**	0.003356	0.002244	-1.4953	0.6688	0.5976

***p >0,05**

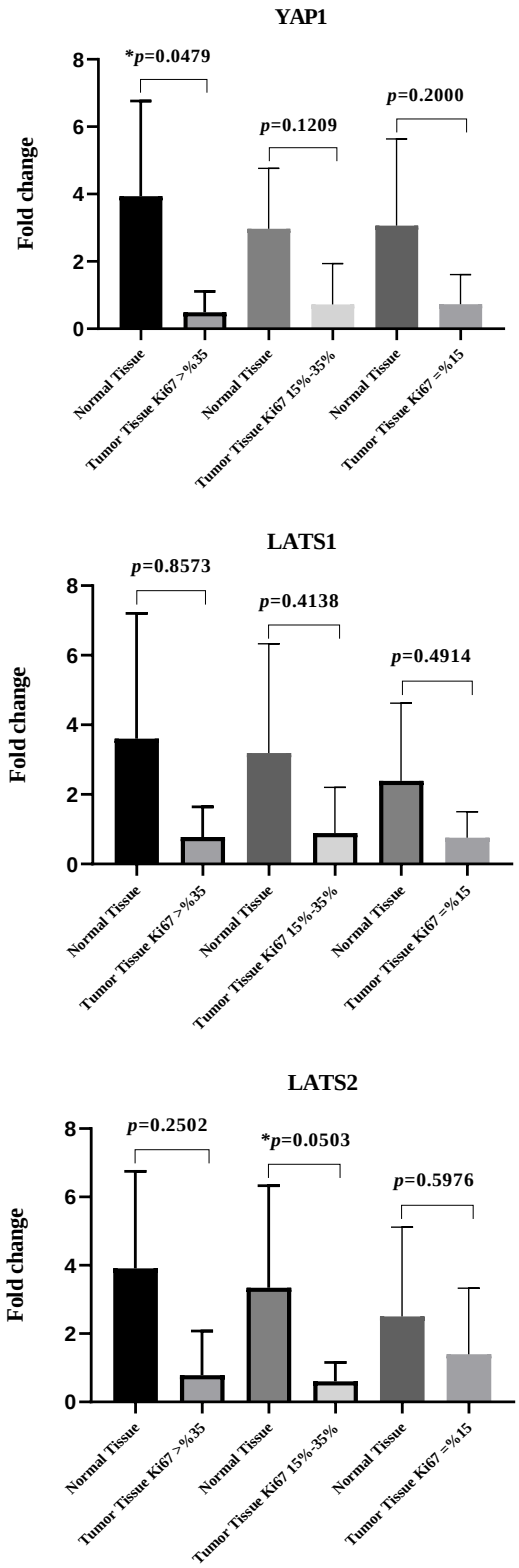


Figure. 21 *YAP1*, *LATS1*, and *LATS2* expressions correlation with the Ki-67 proliferation index.

4.10 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with Lymph nodes

Statistical analysis showed that levels of low-expression of *YAP1* were significantly correlated with Lymph node (Lymph nodes; 1–3 nodes) and (Lymph nodes; at least 4 nodes) (p=0,0144, p=0,0342; respectively). Also, *LATS2* were significantly correlated with Lymph nodes at least 4 nodes (p=0,0237) (Table 18, Figure 22).

Table. 18 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with Lymph nodes

Gene	Normal Tissue	Tumor Tissue (Lymph nodes; 1–3 nodes) n= 27				Normal Tissue	Tumor Tissue (Lymph nodes; at least 4 nodes) n= 19				Normal Tissue	Tumor Tissue (Lymph nodes; node-negative) n= 48			
	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value	2^(Avg.(Delta (Ct))	2^(Avg.(Delta (Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.021939	0.006415	-3.420	0.2924	**0.0144**	0.02296	0.008618	-2.664	0.3754	**0.0342**	0.019032	0.008642	-2.2022	0.4541	0.4229
LATS1	0.023604	0.008412	-2.806	0.3564	0.6203	0.036651	0.012462	-2.941	0.34	0.4129	0.028632	0.017917	-1.598	0.6258	0.6079
LATS2	0.0034	0.001135	-2.9955	0.3338	0.1798	0.002862	0.001228	-2.3295	0.4293	**0.0237**	0.002529	0.001695	-1.4917	0.6704	0.7453

***p <0,05**

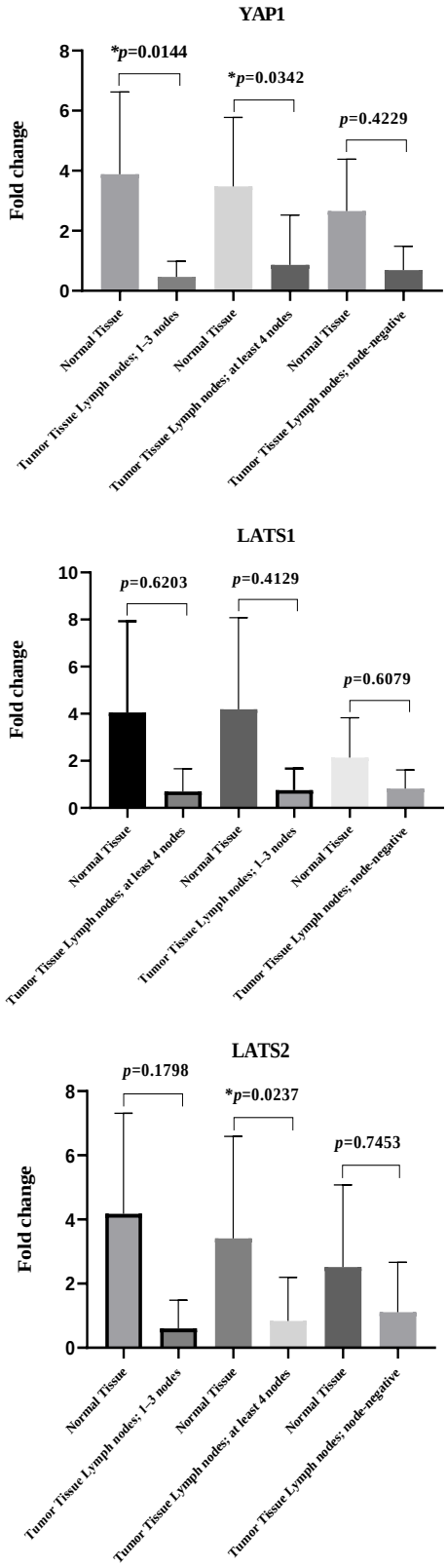


Figure. 22 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with Lymph nodes

4.11 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with *ER-, PR-, HER2-* (TNBC)

Statistical analysis demonstrated that levels of low-expression of *YAP1* were significantly correlated with TNBC tumors (*ER-*, *PR-*, *HER2-*) (*p*=0,0251) (Table 19, Figure 23). Also *LATS1/2* downregulated in TNBC tumors, whereas statistically no had significantly associated.

Table. 19 The Relationship between *YAP1, LATS1* and *LATS2* Expressions with *ER-, PR-, HER2-* (TNBC)

Gene	Normal Tissue	Tumor Tissue (TNBC; ER-, PR-, HER2-) n= 6			
	2^(Avg.(Delta(Ct))	2^(Avg.(Delta(Ct))	Fold Regulation	Fold Change	*p*-value
YAP1	0.022956	0.006368	-3.605	0.2774	***0.0251**
LATS1	0.201894	0.062212	-3.2453	0.3081	0.1986
LATS2	0.001935	0.000942	-2.0539	0.4869	0.1397

***p <0,05**

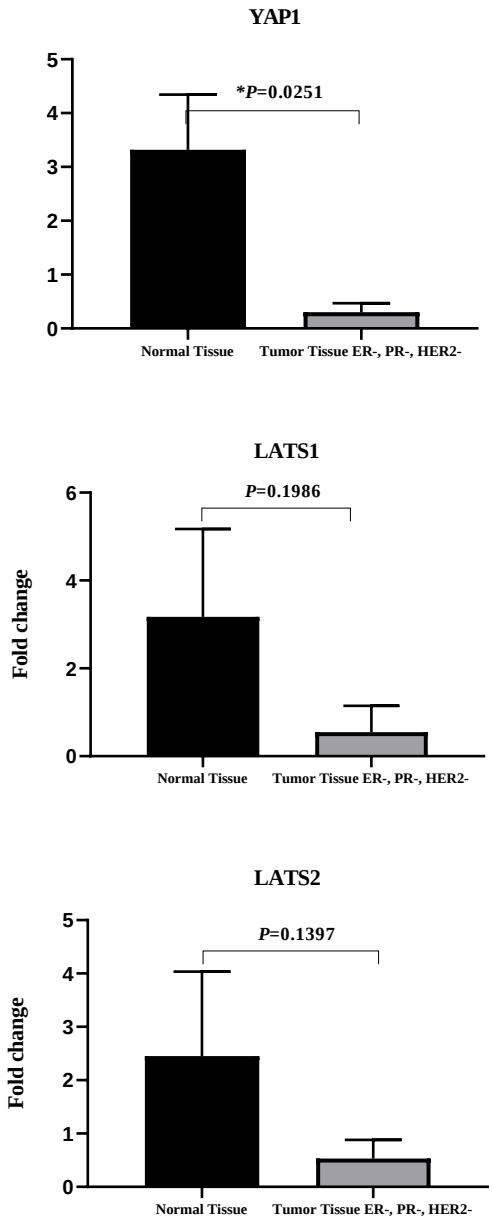
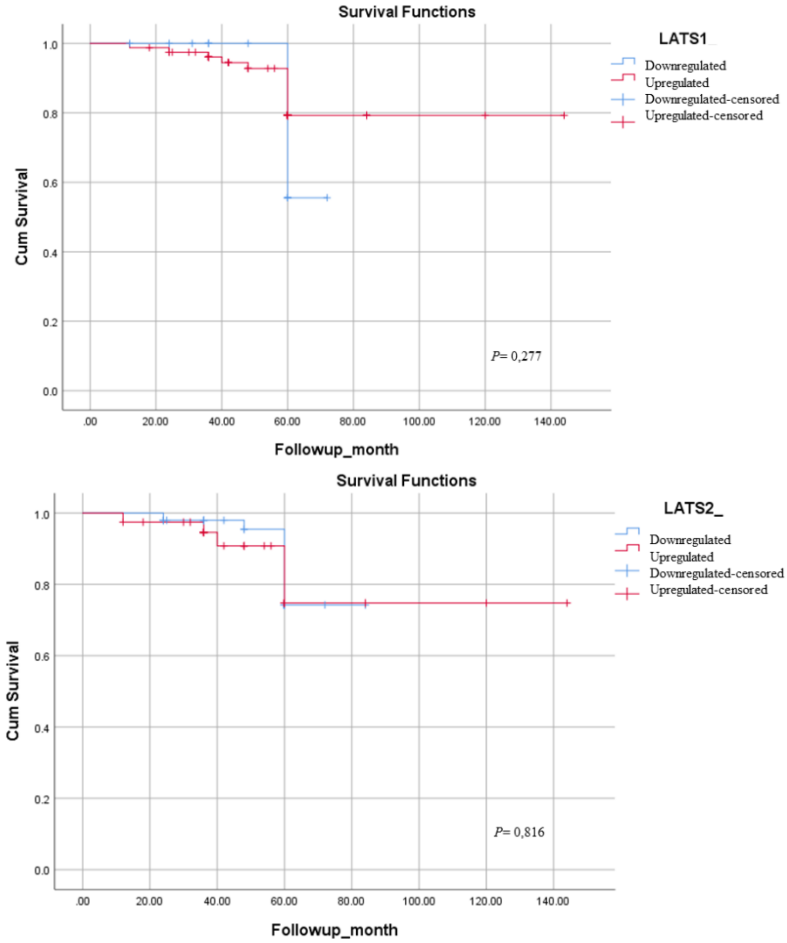


Figure. 23 The Relationship between *YAP1*, *LATS1* and *LATS2* Expressions with *ER-*, *PR-*, *HER2-* (TNBC)

4.12 Effect of *YAP, LATS1* and *LATS2* mRNA Expression on Overall Survival (OS) in IDC Breast Cancer

The follow-up time range of breast cancer patients was a mean 60 months. The Kaplan–Meier analysis of overall survival in IDC breast cancers for *YAP*, *LATS1* and *LATS2* expression and clinicopathological features was made. In this study, *YAP, LATS1*, and *LATS2* mRNA expressions no significantly had in overall survival of the patient (Figure 18).



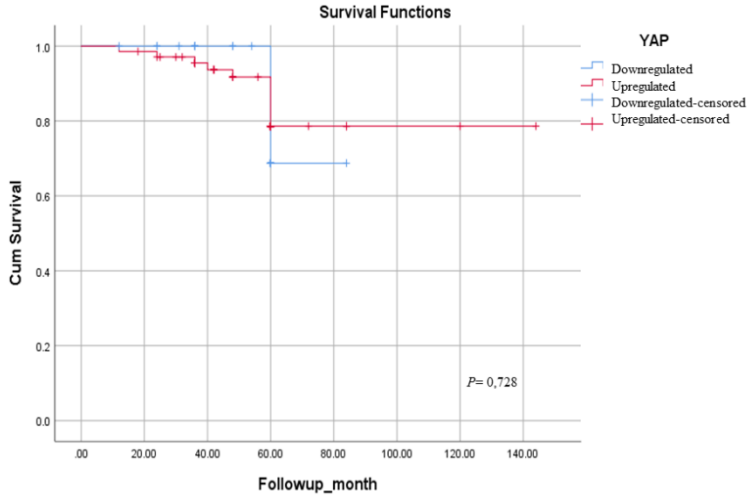


Figure. 24 Effect of *YAP*, *LATS1*, and *LATS2* mRNA expression on overall survival (OS) in IDC breast cancer

5. DISCUSSION and CONCLUSION

Breast cancer is a heterogeneous disease and differs greatly among different patients and even within each individual tumor (Ellsworth et al., 2017). Heterogeneity in the expression of established prognostic and predictive biomarkers, hormone receptors, and human epidermal growth factor receptor 2 oncoprotein is the basis for targeted treatment. Invasive ductal carcinoma (IDC) is the most common form of breast cancer, comprising 70% to 80% of all breast cancers. Molecular classifications are indicators of genetic tumor heterogeneity, which is probed with multigene assays and can lead to improved stratification into low- and high-risk groups for personalized therapy, and understanding the molecular and cellular mechanisms of tumor heterogeneity that are relevant to the development of treatment resistance is a major area of research (Li et al., 2005; Turashvili et al., 2017). Recent advancements in the field of oncology have led to the discovery of several complex and functionally diverse signaling pathways implicated in breast cancer metastasis. The Hippo pathway is a novel and highly conserved mammalian signaling pathway. Mutations and altered expression of core Hippo pathway components (*YAP1, TAZ, LATS1, LATS2, MST1,* and *MST2*) promote the migration, invasion, malignancy, and chemotherapy resistance of breast cancer cells. In cancer metastasis, tumor cells must detach from the primary tumor, invade surrounding tissue, and enter and survive in a foreign microenvironment. The metastatic potential of breast cancer is closely related to the individual patient's genetic profile (Li et al., 2005; Moroishi et al., 2015; Varelas et al., 2014). In the present study, the Hippo pathway component levels in 100 patients with IDC breast cancer with a mean follow-up period of 60 months were analyzed, and the association of Hippo pathway components levels with

survival and clinicopathological characteristics of patients were assessed. The major effectors of the core component in the Hippo pathway are *YAP* and *TAZ. TAZ* is overexpressed in 20% of breast cancers (Chan et al., 2008), and *TAZ* expression levels and activity are frequently upregulated in high-grade metastatic breast cancer (Cordenonsi et al., 2011). In IDC breast tumor tissues analyzed in our study with RT-PCR, *YAP* expression significantly in TNBC tumors was down-regulated. A meta-analysis demonstrated a prognostic significance of Hippo pathway components in overall survival (OS) and its association with clinicopathologic characteristics especially, TNM stage (Feng et al., 2016). In the present study, deregulated *YAP*, *LATS1*, and *LATS2* expression no had a significant association with the overall survival (OS).

Previous reports propose that *YAP* can behave as either an oncoprotein or tumor suppressor in different cellular contexts. Consistent with its role as a tumor suppressor, the level of *YAP* was decreased or lost in breast cancers and its knockdown in breast cancer cell lines suppressed anoikis and increased migration and invasion (Yuan et al., 2008). Exchanging binding partners from TEAD to *RUNX3* can shift the role of *YAP* from oncoprotein to tumor suppressor. Also, Lin et al. showed that environmental stress promotes the cytoplasmic translocation of *TEAD*, which selectively suppresses the oncogenic activity of *YAP* (Lin et al., 2017). Overall, the activity of Hippo signaling may be different in different cellular context. In our study, significantly *YAP1* expression was downregulated, this was similar to the study of Box-and-Whisker plots that was showed the expression of *YAP* mRNA was significantly lower in tumor tissues breast cancer (Real et al., 2018). Also in study Box-and-Whisker et al, a significant association was observed on correlating mRNA expression of *YAP1* with clinical stages and *ER* status among breast cancer patients. In this present study, a significant association was revealed on correlating mRNA expression of *YAP1* with *PR* status, tumor size (>3 cm), *HER2*-positive, metastasis tumors, Ki-67 proliferation index. As well as in our study a significant association observed between *YAP1* and lymph nodes. Guo et al indicated greater expression of *YAP1* mRNA in breast cancer tissues than in normal breast tissues and a negative correlation with patient survival (Guo et al., 2019), that their results were reversed with our results and in our study no significant association revealed on

overall survival and low-level of *YAP1*. *LATS1* and *LATS2* are introduced as redundant paralogs that phosphorylate and inactivate the *YAP* and *TAZ* and been implicated in modulating ER protein stability (Britschgi et al, 2017; Moroishi et al., 2015). Furth et al showed that *LATS1* and *LATS2* are down-regulated in human breast cancer. Yet, evidence of distinct functions and differential impacts of the two paralogs is accumulating (Furth., 2017). In a study by Furth et al in the vivo breast cancer setting evidence provided that both *LATS1* and *LATS2* are tumor suppressors, and in a luminal B breast cancer mouse model, conditional deletion of either paralog increases tumorigenesis, both in magnitude and severity (Furth et al., 2018). Evidence surrounding the clinical implications of the Hippo pathway in breast cancer cases is scarce, and little is known about how the underlying molecular mechanisms of this pathway are regulated. In this study, we showed that *LATS1* and *LATS2* down-regulated in IDC patients and low-level of *LATS2* with special attention given to its relationship with the clinical features of breast cancer metastasis and also, in our study a significant association was observed between *LATS2* and lymph node, and ki-67 proliferation index. Li et al have shown that lack of *LATS* increase the stability of *ERα* and *YAP/TAZ*, suggesting that the Hippo pathway may also be able to regulate the fate of breast cell. Decreased *LATS* levels in patients are associated with reduced relapse-free survival (Li et al., 2014). A study has found that the decreased expression of *LATS1* or *LATS2* mRNA in breast cancer tissues leads to increased tumor size and lymph node metastasis and is negatively related to the presence of estrogen and progesterone receptors (Visser et al., 2010), in our study, low-level *LATS1/LATS2* associated with tumor size and tumor grade, but not statistically significant. Mammalian sterile 20-like kinase 1 *(MST1)* is a major inhibitor of cell proliferation and is involved in apoptosis, oncogenesis and organ growth via its ubiquitously encoded serine-threonine kinase (Song et al., 2010). Previous studies have demonstrated that *MST1* has a tumor suppressor function in human breast cancer. *MST1* deletion or mutation is associated with tumorigenesis, whereas *MST1* overexpression leads to tumor cell apoptosis and decreases the proliferation of tumor cells. The previous study reported the tumor-suppressive function of *MST1* and debated *MST1* as a prognostic factor in human breast cancer. *MST1,* act as tumor suppressors that regulate *YAP* phosphorylation (Lin et al., 2017). However, less is

known about the role of *MST* in breast cancer metastasis. In the present study, *MST1* levels were measured in the tumor tissues of patients in order to elucidate their association with clinicopathologic features. The results of the present study indicated that *MST1* is downregulated in tumor tissues. Rauch et al, important of the pro-apoptotic mammalian sterile 20-like kinase *(MST2)* tumor suppressor in several cancer entities, including head and neck, colon, and breast demonstrated. But, less is known about the role of *MST2* in breast cancer metastasis and there is no study about the relation to clinicopathologic features. In our study, the *MST2* levels of IDC breast cancer tissues analyzed, and *MST2* was downregulated but this was not statistically significant. In breast cancer, ductal carcinoma in situ (DCIS) is recognized as a precursor of invasive ductal carcinoma (IDC) and usually accompanies IDC, which is associated with different clinical courses and treatment strategies. Breast IDC is assumed to appear de novo in patients with pure IDC; in patients with IDC with DCIS component (IDC- DCIS), it is postulated that IDC develops from a pre-existing DCIS lesion. studies have found that breast IDC-DCIS is associated with higher rates of local recurrence due to a higher incidence of positive surgical margins. In addition, patients with breast IDC-DCIS had better clinical outcomes compared with patients with pure IDC of the breast (Wu et al., 2018). In our study, low level of YAP1 significantly was associated with in situ component- negative in IDC breast cancer.

In conclusion, the Hippo pathway plays an undeniably important role in breast cancer, based on converging evidence from both clinical and experimental investigations. The future of breast cancer metastasis management would benefit tremendously from additional scrutinization of the Hippo pathway, which will pave the way for the development of more accurate prognosticating markers and treatment targets. Thus, the components of the Hippo pathway can serve as predictive biomarkers and as therapeutic targets for the treatment and management of breast cancer. The observations from all studies and our results support *YAP* to be a tumor suppressor gene and also to be as a theraputic of goals for breast cancer pateints. To the best of our knowledge, we are the first examined all component hippo pathway and their association with clinicopathologic features in IDC breast cancer patients.

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7. ABBREVIATIONS

- World Health Organization (WHO)
- Invasive ductal carcinoma (IDC)
- Ductal Carcinoma in situ (DCIS)
- Lobular Carcinoma in situ (LCIS)
- Invasive Lobular Carcinoma (ILC)
- Immunohistochemistry (IHC)
- Estrogen Receptor (ER)
- Progesterone Receptor (PR)
- Human Epidermal Receptor (HER)
- Triple-Negative Breast Cancers (TNBC)
- Androgen receptor (AR)
- Musculoaponeurotic fibrosarcoma (c-MAF)
- Cellular myelocytomatosis (c-MYC)
- Cysteine-rich angiogenic factor (CYR61)
- Extracellular matrix (ECM)
- US food and drug administration (FDA)
- Glucocorticoid receptor (GR)
- Large tumor suppressor 1/2 (LATS1/2)
- MAP kinase kinase kinase kinases (MAP4K)
- MOB kinase activator 1 (MOB1)
- Mammalian Hippo homolog (Ste20-like kinases) (MST1/2)
- Mammalian target of rapamycin (mTOR)
- Phosphoinositide 3-kinase/protein kinase A (PI3KCA)
- Phosphatase and tensin homolog (PTEN)
- Rapidly accelerated fibrosarcoma family of serine/threonine kinases (RAF)
- Rho family of GTPases (RhoGAP)

- Protein salvador homolog 1 (SAV1)
- Transcriptional co-activator with PDZ-binding motif (TAZ)
- TEA domain family member 1–4 (TEAD1-4)
- Wingless (WNT)
- Yes-associated protein (YAP)
- Epidermal Growth Factor Receptors (EGFR)
- Mitogen-Activated Protein Kinase (MAPK)
- American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP)
- Epithelial to Mesenchymal Transition (EMT)
- Formalin-fixed, Paraffin-embedded (FFPE)
- Cycle threshold (Ct)
- RT- qPCR (Real-Time quantitative reverse transcription PCR)
- Vascular Endothelial Growth Factor (VEGF)
- Oncogenic c-Terminal Cyclin D1 (CCND1)
- Glycogen Synthase Kinase-3 (GSK3)
- Nuclear Factor of Activated T Cells 5 (NFAT5)
- Angiomotin-Like 1 (AMOTL1)
- AMP-Activated Protein Kinase (AMPK)
- Abelson Tyrosine Protein Kinases (ABL)
- Epidermal Growth Factor (EGF)
- Proline-Proline-Any Amino Acid-Tyrosine (PPXY)
- Krueppel-Like Factor 5 (KLF5)

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9. CURRICULUM VITAE

Maryam SABOUR TAKANLOU was born on the 14rd of January of 1990 in Tabriz, Iran. She was accepted in the Faculty of Science of the University of Islamic Azad, Tabriz, Iran where she studied in the field of Cellular and Molecular Biology-Genetic and has conferred a degree of Bachelors of Science in August of 2013.

She was then accepted in the faculty of graduate studies in the Department of Cellular and Molecular Biology- Genetic, University of Islamic Azad, Tabriz, Iran for the Master's program in August of 2016. The title of her thesis was Cytogenetic and molecular studies of Y chromosome microdeletions in AZFa, AZFb, and AZFd gene deletions in infertile men in Northwest of Iran.

In February 2017, she was accepted as a Master in Bursa Uludag University, Bursa, Turkey and She is continuing her master's degree in 2019 at Bursa Uludag University, Faculty of Medicine, Department of Medical Biology.