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The rictor phosohorylation is growth factor dependent in the breast cancer cells

Abstract: In spite of advances in early diagnosis breast cancer remains a dreadful disease, approximately 40,000 women die every year. Deregulation of growth factor signaling has been assigned as a hallmark in cancer development and progression. A multi-step process driven by a highly selective pressure takes place in transformation of mammary epithelial cells and development of malignant phenotype. Activation of cellular signaling pathways engaged in regulation of cell proliferation, survival, and migration is a key factor in tumorigenesis. In this regard, one of the most frequently activated signaling component in human cancer is Akt that provides leverage for cancer cells in up-regulation of cellular proliferation and survival required for sustained tumor growth. In our previous work we have identified mammalian Target of Rapamycin Complex 2 (mTORC2) as a crucial regulator of Akt. An important role of the novel mTORC2 complex as a regulator of Akt attracts a great interest in cancer research. The central hypothesis of this study is that during cellular transformation the mTORC2 signaling is up-regulated to activate the Akt pathway as an important step in development of primary breast cancer tumors. According our study, the development of the novel breast cancer marker based on indirect evaluation of the mTORC2 activity is feasible. Besides, it will be highly valuable to develop a specific inhibitor of mTORC2 to target the breast cancer linked to hyperactivation of Akt. The study of mTORC2 regulation is a promising area of research for development the breast cancer therapeutics. The novel breast cancer marker will identify the mTORC2-dependent types of breast cancers and be applied for early diagnostics and characterization of breast cancer. Development of a specific inhibitor of mTORC2 will provide the specific targeting tool to suppress the growth factor signaling in breast cancer.

Keywords: rictor, breast cancer, mTOR, mTORC1, mTORC2, insulin-like growth factor I

A. Background. A-1. Hyperactivation of the Akt signaling in breast cancer. In spite of advances in early diagnosis breast cancer remains a dreadful disease: approximately 40,000 women die of the disease every year (Greenlee et al., 2000). Deregulation of growth factor signaling is a hallmark in cancer development and progression. The insulin-like growth factor (IGF) or epidermal growth factor (EGF) tyrosine kinase signaling pathways are commonly deregulated pathways in human breast cancer (Klijn et al., 1992; Slamon et al., 1989; Surmacz, 2000; Zhang and Yee, 2000). Amplification of the growth factor-dependent ras and phosphatidylinositol-3-OH kinase (PI3K) pathways leads to hyperactivation of the Akt signaling, which provides leverage for cancer cells in the up-regulation of cellular proliferation and survival required for sustained tumor growth (Brognard et al., 2001; Hutchinson et al., 2004; Shaw and Cantley, 2006) . The IGFI expression has been shown to be associated with most human breast cancers (Surmacz, 2000). Overexpression of HER2 is associated with tumorigenesis and amplified HER2 has been identified in 20% -30% of breast cancers (van de Vijver et al., 1988). Mutations in ras genes are not common in breast cancer but the highly active ras proteins have been identified in approximately 50% of the studied breast tumors (von Lintig et al., 2000), most likely linked to up-regulation of growth factor signaling. The clustered regions of point mutations have been revealed in the p110a catalytic subunit of PI3K in 20%-30% of the breast, colon, brain, and gastric tumors examined (Samuels et al., 2004). In addition, a loss of PTEN is the most common mechanism of PI3K activation in human cancers. PTEN lipid phosphatase acts as an enzyme to reverse the kinase reaction catalized by PI3K. PTEN catalizes removal of the D3 phosphate from phosphatidylinositol-3,4,5-triphosphates and thereby counterbalances the PI3K signaling (Maehama and Dixon, 1998). A large number of sporadic mutations of PTEN are identified in a high level in many tumor types, including breast, ovarian, and colon cancers and

glioblastoma, defining PTEN as the second most commonly mutated tumor suppressor gene after p53 (Shaw and Cantley, 2006).

A role of Akt in cancer development associated with its hyperactivation and phosphorylation on the Ser-473 site has been intensively studied in various human cancers (Balsara et al., 2004; Kreisberg et al., 2004; Sun et al., 2001; Tsurutani et al., 2006), and it has been found in more than 40% of breast cancers that correlated with disease progression from abnormal hyperplasia to tumor invasion (Zhou et al., 2004). Akt is the evolutionarily conserved serine/threonine kinase and an essential downstream effector of the PI3K pathway in growth factor signaling. Akt is represented by subfamily of kinases containing three isoforms, Akt1, Akt2, and Akt3. The Akt kinases are members of the AGC (protein kinase A, G, and C) kinase family that act on a wide spectrum of substrates (Bellacosa et al., 2005). The Akt signaling is engaged in multiple signaling pathways and involved in regulation of a various of cellular processes, including cell proliferation, growth, survival, and metabolism. We only recently identified mTORC2 (mammalian Target Of Rapamycin Complex 2) as a long sought Ser-473 kinase of Akt (Sarbassov et al., 2005). An important role of the novel mTORC2 complex as a regulator of Akt attracts a great interest in cancer research.

A-2 The mTORC2 complex regulates Akt by phosphorylating its Ser-473 site. The Akt kinase is a well-characterized essential effector of PI3K in growth factor signaling. Activation of PI3K results in accumulation of phosphatidylinositol-3,4,5-triphosphates (PIP3s) which initiate recruitment of Akt to plasma membrane by its plekstrin homology domain. At this location Akt is phosphorylated on the Thr-308 and Ser-473 sites required to fully activate Akt (Bellacosa et al., 2005). The activation loop of Akt on Thr-308 is phosphorylated by the phosphoinositide-dependent kinase 1 (PDK1) that required for the kinase activity of Akt (Alessi et al., 1997) (Stephens et al., 1998). PDK1 was identified in 1997, but the Ser-473 kinase of Akt, named PDK2, which was sought for many ears, was only recently identified as the mTOR Complex 2 (mTORC2) (Sarbassov et al., 2005). Although several candidates were proposed earlier as PDK2 (Dillon et al., 2007), the mouse genetic studies confirmed the role of mTORC2 as the Ser-473 kinase of Akt (Guertin et al., 2006; Jacinto et al., 2006; Shiota et al., 2006; Yang et al., 2006). This phosphorylation event is coupled to activation of Akt and it is involved in regulation of the PDK1-dependent phosphorylation on Thr-308 of Akt by facilitating recognition of Akt by PDK1 (Scheid et al., 2002; Yang et al., 2002).

The mTORC2 complex is assembled by the mTOR interacting proteins rictor, Sin1, and mLST8 (Frias et al., 2006; Jacinto et al., 2006; Jacinto et al., 2004; Sarbassov et al., 2004) . mTORC2 as a regulator of Akt, an essential kinase downstream of PI-3 kinase, expands mTOR's function to regulation of cellular proliferation, survival, motility, and metabolism. A role of mTORC2 in regulating of PKCa and the cytoskeleton has also been reported (Jacinto et al., 2004; Sarbassov et al., 2004). A central component of this complex is mTOR, an essential and highly conserved protein. It contains multiple HEAT repeats at the N-terminal half of the protein that are known to form a scaffolding structure for protein/protein interactions. The FRB domain responsible for binding of the rapamycin/FKBP12 complex is a stretch of 100 amino acids located in the C-terminal half of mTOR. The mTOR kinase domain is located at the C-terminus, structurally resembles a kinase domain of PI3K but functions as a serine/threonine protein kinase and it is essential for mTOR's function (Harris and Lawrence, 2003). In contrast to mTORC1, mTORC2 does not bind the rapamycin/FKBP12 complex, suggesting that the FRB domain on mTOR that is responsible for the binding is not accessible on mTORC2. Nevertheless, prolonged rapamycin treatment causes an indirect effect on mTORC2 by inhibiting the assembly of this complex. In some cell types, mostly lymphoma cells, the prolonged rapamycin treatment causes inhibition of Akt because of a dramatic effect on the abundance of mTORC2 (Sarbassov et al., 2006). Within the mTORC2 complex, mLST8 also known as GbL, a small adaptor protein containing seven WD40 repeats, binds tightly to the kinase domain of mTOR (Jacinto et al., 2004; Sarbassov et al., 2004). Binding of mLST8 to mTOR is required for the kinase activity of mTOR (Kim et al., 2003). Another mTORC2 component rictor forms a low affinity complex with mTOR and it is indispensable for the function of mTORC2. Rictor remains poorly characterized. The full length of the human rictor polypeptide containing 1,708 amino acids reveals no homology with any known functional domain or protein, although it is relatively conserved in all eukaryotes (Sarbassov et al., 2004; Sarbassov et al., 2005). Initially,

rictor's ortholog, *pianissimo,* was identified in *Dictyostelium* as a critical player in chemotaxis and cAMP signal relay (Chen et al., 1997). The recently identified fourth component of mTORC2, Sin1, might provide more insights into the regulation and function of mTORC2. Two Sin1 functional domains have been proposed: the Raf-like Ras binding domain (RBD) and a pleckstrin homology domain (Schroder et al., 2007). The RBD domain points out Ras as a potential up-stream effector of mTORC2, and localization of mTORC2 at the plasma membrane might depend on the plekstrin homology domain of Sin1. Like rictor's, Sin1's ortholog was initially identified as an important regulator of chemotaxis and in addition as a Ras interacting protein 3 in *Dictyostelium* (Lee et al., 2005; Lee et al., 1999). In this proposed project, we wexamined the role of PI3K in regulation of the mTORC2 activity.

B. Results B-1 Rictor phosphorylation is linked to regulation of the mTORC2 kinase activity. To study growth factor dependent activation of mTORC2, we examined the IGFI-dependent phosphorylation of its substrate Akt in three breast cancer cell lines, MDA-MB-231, MCF7, and MDA-MB-435. We found that the IGFI stimulation of the serum-starved cells induced a robust phosphorylation of Akt on the Ser-473 site in all three breast cancer cell lines (Fig. 1A and B). It indicates that all three cancer cell lines are highly sensitive to the IGFI stimulation that associates with the phosphorylation of the mTORC2 substrate Akt . We also observed the change of rictor's mobility in a gel. In our initial characterization of rictor we have shown that rictor's mobility is depend on its phosphorylation state (Sarbassov et al., 2004). Taken together, these data suggest that the IGFI-dependent activation of the mTORC2 function is linked to phosphorylation its component rictor. It is possible that in active state of mTORC2, mTOR acts as a rictor kinase and the phosphorylation of rictor takes place within the mTORC2 complex. In the next experiment we addressed whether the activation of mTORC2 takes place following the stimulation of cells with IGFI by performing in vitro mTORC2 kinase assay as described previously (Sarbassov et al., 2005). We detected a greater kinase activity in the mTORC2 sample purified from the IGFI stimulated cells than in the sample purified from serum starved cells (Fig. 1B, the upper panel). Thus, the growth factor-dependent phosphorylation of rictor is linked to activation of the kinase activity of mTORC2.

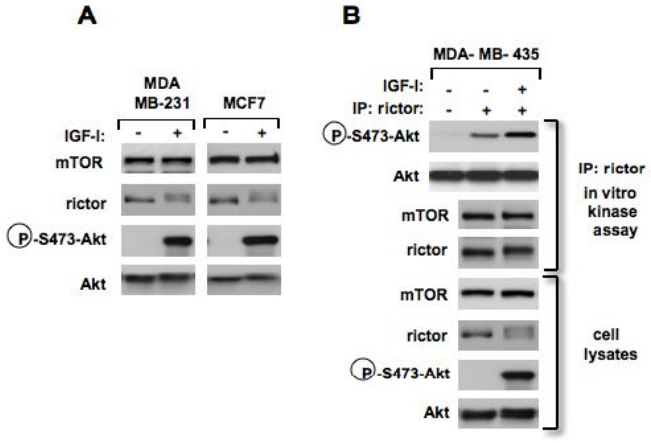

Figure 1 – Rictor phosphorylation associates with the IGFI-dependent stimulation of the mTORC2 kinase activity **A.** The IGFI stimulation causes a slower mobility of rictor protein. The serum starved MDA-MB-231 and MCF7 cells were stimulated by IGFI. The lysates were analyzed by immunoblotting for indicated proteins and phosphorylation of Akt. **B.** The IGFI stimulation induced the phosphorylation of rictor and a kinase activity of mTORC2. The rictor immunoprecipitates from the serum starved or IGFI stimulated MDA-MB-435 cells were used in a kinase assay with a full-length wild type Akt1 as a substrate. Immunoblotting was used to detect the phosphorylation of Akt on Ser-473 and the amount of mTOR, rictor, and Akt1 in the kinase assay. Akt1 itself without immunoprecipitate was used in a kinase assay to detect a basal phosphorylation of the substrate (upper panel). The MDA MB 435 cell lysates were analyzed in parallel as described in A (lower panel).

B-2 The IGFI-dependent phosphorylation sites of the mTORC2 component rictor. Regulation of the mTORC2 activity by IGFI is linked to phosphorylation of its component rictor, although the function of this phosphorylation in the mTORC2 signaling is unknown. Identification of the phosphorylation sites on rictor is a critical step in addressing this lack of knowledge. This project was initiated by the mass spectrometry analysis of the immunopurified rictor samples from

the stimulated HeLa cells. We selected several rictor peptides as potential carriers of phosphorylation sites on the basis of their distinct mass readings. To study these potential phosphorylation sites of rictor, we initiated development of the rictor phospho-specific antibodies. The first three antibodies that recognize the rictor phosphorylation sites on Thr-1135, Thr-1177, and Ser-1219 were recently developed and validated.

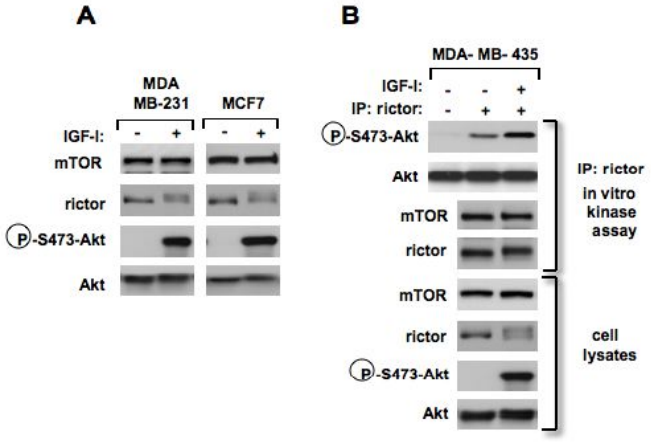

Figure 2 – Detection of the IGFI dependent phosphorylation sites of the mTORC2 component rictor
The serum starved MDA-MB-435 cells with or without the IGFI stimulation were lysed and analyzed by immunoblotting for the indicated proteins and phosphorylation states by the phospo-specific rictor and Akt antibodies. Where indicated the cells were also pre-incubated with LY294002 at 20 micromolar concentration for 24 hrs (left panel). In parallel, in the similar conditions the mTOR bound rictor phosphorylations were analyzed in the mTOR immunoprecipitates as shown in a right panel.

We found that a detection of rictor by the rictor phospho-Thr-1135 and Ser-1219 antibodies was greater in the cellular extracts of MDA-MB-435 cells stimulated by IGFI than it was those of unstimulated serum-starved cells (Fig. 2, a left panel). This finding confirmed that the rictor phosphorylation at least on Thr-1135 and Ser-1219 sites takes place following stimulation of cells with IGFI. However the signal detected by the rictor phospho-Thr-1177 antibody showed no increase following IGFI stimulation, suggesting either constitutive phosphorylation of this site or that the antibody binding to rictor is independent of the phosphorylation of this site. Validation of this rictor site will be undertaken with a different batch of the antibody. As also shown in Fig. 2 (left panel), a pre-incubation of cells for 16 hrs with the PI3K inhibitor LY294002 (also known as inhibitor of the mTOR kinase activity) caused a decrease of the basal signal detected by these phospho-specific rictor antibodies under unstimulated conditions. This implies that under serum-starved condition, PI3K or mTOR carries sufficient basal activity to sustain a low level of rictor phosphorylation. Most importantly, the pre-incubation of cells with LY294002 inhibited the IGFI induced phosphorylation of rictor on Thr-1135 and Ser-1219 indicating that PI3K or mTOR plays a role in this phosphorylation event.

After studying rictor phosphorylation in total cellular lysates, we analyzed it in association with the mTORC2 complex. We purified mTORC2 by immunoprecipitating mTOR from cells in experimental conditions similar to those described above by lysing cells in a buffer containing a mild detergent, CHAPS, to preserve the complex. We found that the mTORC2 component rictor was phosphorylated in response to the IGFI stimulation on Thr-1135 and Ser-1219 sites and this phosphorylation was sensitive the pre-incubation of cells to LY294002 (Fig. 2, a right panel). We found a similar pattern of the rictor phosphorylation in the total cellular extracts and the immunopurified mTORC2. Thus, the rictor phosphorylation in total cellular extracts indicates the rictor phosphorylation within the mTORC2 complex and can be applied as the indirect activity marker of this kinase complex. Also, in the same samples of total cellular lysates and immunopurified mTOR we also detected the growth factor-dependent changes in mobility of the Sin1 protein. It implies a role of phosphorylation of Sin1 in growth factor-dependent regulation of mTORC2.

C. Discussion and future directions. Deregulation of growth factor signaling is a hallmark in cancer development and progression. The IGF or EGF signaling pathways are commonly deregulated pathways in human breast cancer. Amplification of the growth factor-dependent ras and

phosphatidylinositol-3-OH kinase (PI3K) pathways leads to hyperactivation of the Akt signaling, which provides leverage for cancer cells in the up-regulation of cellular proliferation and survival required for sustained tumor growth. The focus of this study is mTORC2, the important player in growth factor signaling with its enzymatic kinase activity that attracts a great interest as a novel anti-cancer drug target.

C-1. Hyperactivation of Akt is common in breast cancer and it is linked to its phosporylation on the regulatory Ser-473 site by mTORC2. As described in the Results (Sections B1 and B2), we have identified the phosphorylation sites of the mTORC2 components rictor and mTOR, and these post-translational modifications are coupled to the functional activity of the mTORC2 complex as the regulatory Ser-473 kinase of Akt. We found that the phosphorylation of rictor on Thr-1135 and Ser-1219 sites takes place in a process of activation of the mTORC2 kinase in breast cancer cells stimulated by IGFI. This finding allows an indirect evaluation of the kinase activity of mTORC2 by applying the phospho-specific rictor and mTOR antibodies validated in this study. The both phosphorylation sites of rictor could serve as the novel markers to assess activity of the mTORC2 kinase activity in breast cancer based on detection of the rictor phosphorylation. It will be a valuable tool to identify and characterize the mTORC2-dependent types of breast cancer.

Development of the novel breast cancer marker based on detection of the mTORC2 activity will be valuable to assess htis regulatory Ser-473 kinase of Akt. Hyperactivation of Akt is associated with variety types of cancer including breast cancer. The high abundance of the mTORC2 components rictor and mTOR in human breast tumors correlates with its role as the regulatory Ser-473 kinase of Akt required for sustained tumor growth. Development of the novel breast cancer marker can be based on detection of the rictor phosphorylation by the phospho-specific rictor antibodies described in Results section B-2. Initially, the study has to determine if the antibodies will recognize their phosphorylation sites on the non-denatured native form of rictor. It is likely that the phospho-specific antibodies will efficiently immunoprecipitate rictor from the IGFI-stimulated but not from the serum-starved cells. The CHAPS lysis buffer will be applied to preserve the mTORC2 complex. If the phosphorylation sites of rictor will not be accessible for the antibodies in the complex, the cells will be lysed in in the Triton lysis buffer known to disintegrate the mTORC2 complex. It might be necessary to validate the phospho-specific rictor antibodies in the immunofluorescence study of the serum-starved or IGFI-stimulated breast cancer cells. If necessary the permeabilization step will be introduced by incubating the fixed cells in a stringent Triton containing buffer. To assure the specificity of staining the knock down of rictor by the retroviral expression of shRNAs targeting rictor mRNA will be instrumental. Extension of the rictor phospho-specific antibody applications to stain breast tumors by immunohistochemistry (IHC) is a critical final step in the marker development. The breast tumor samples for IHC can be first obtained from the mouse mammary tumor virus (MMTV)-HER2 transgenic mice to determine a feasibility of the phosphor-rictor antibodies for detection the HER-dependent mTORC2 activation. After the validation of the phospho-specific rictor antibodies in the IHC staining of the mouse tumor samples, it will be feasible to initiate the staining of human breast tumors.

C-2. An important role of mTORC2 as regulator of Akt attracts a lot of attention and translates mTORC2 to a highly attractive anti-cancer drug target, particular for cancer types with the hyper-activated Akt pathway. It correlates well with our finding that demonstrates the up-regulation of the mTORC2 components rictor and mTOR in human breast tumors as shown in the Results section B-3. In our previous work we found that a prolonged rapamycin treatment causes only a partial inibition of the mTORC2 assembly and in most of the cancer cells the Akt pathway remains insensitive to rapamycin treatment. It can be predicted that active compounds affecting the mTORC2 function in combination with rapamycin will have a synergistic impact on cancer cells linked to the potent inhibition of Akt. Our study incites a development of the mTORC2 inhibitor that will have a great potential in translational research.

Targeting the mTORC2 complex in breast cancer by developing of its specific inhibitor is a promising approach to suppress breast tumorigenesis. The National Cancer Institute library of natural chemical compounds will be valuable to carry out the screening studies. The different types of high trough-put screening (HTS) assays are necessary to develop a specific and potent inhibitor

of mTORC2. First, the screening for the mTORC2 kinase activity can be analyzed by detection of the rictor phosphorylation in a cell-based assay. The rictor phospho-specific antibodies described in this study will provide the functional read-out of the mTORC2 activity. Second, it will be practical to perform the screen to identify the synergistic cell toxicity effect in combination with rapamycin. It has been shown in our previous study that a prolonged rapamycin treatment inhibits assembly of mTORC2 and a compound inhibiting mTORC2 in combination with rapamycin will be highly effective in suppressing mTORC2 and cell survival by mimicking a loss of mTORC2. Third, the HTS assay for the compounds causing effect on integrity and assembly of mTORC2 will provide an additional approach to search for the potent inhibitors of mTORC2. This screening approach will be based the mTORC2 purification and analyzing its integrity. The affinity purification of mTOR allows to analyze integrity of both mTORC1 and mTORC2 by detecting abundance of bound raptor or rictor to mTOR. Identification of compound with a specific and selective effect on integrity of the mTORC2 but not to the mTORC1 will be a main interest in this screening approach. A highly quantitative pulse and chase experiment can be applied to study effect of inhibitory compounds on assembly of mTORC2. Most importantly, a mechanism of direct targeting and binding of inhibitory compounds to the mTORC2 will validate the screening studies by co-purification experiments of the mTORC2 components with radiolabeled inhibitory compounds.

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Риктордың фосфорлануы сүт безі қатерлі ісігінің өсу факторларына байланысты

Аннотация Ерте диагностика әдістерінің жетілуіне қарамастан, сүт безі қатерлі ісігі әлі де жылына шамамен 40 000 әйелдің өліміне себепші ауыр дерт болып саналады. Ісіктің туындауы мен дамуы өсу факторлары сигналдық жолдарының бақылаудан шығуымен сипатталады. Сүт безі эпителий клеткаларының трансформациясы жңне қатерлі ісік фенотипінің дамуы жоғары селективті қысымға негізделген көп сатылы процесс болып табылады. Ісік түзілуінің басты факторы болып клеткалардың пролиферациясы, өміршеңдігі және миграциясын реттеуге қатысатын клеткалық сигналдық жолдардың активтенуі саналады. Осыған байланысты, ісік т үзілуі барысында адамда жиі активтенетін сигналдық компоненттердің бірі бұл Akt. Ол ісіктің ұзақ мерзімді өсуіне қажетті ісік клеткаларының пролиферациясы және өміршеңдігі механизмдерінің қосылуын қамтамасыз етеді. Алдыңғы жұмыста біз сүтқоректілердің рапамицин нысаны 2-кешені (Target of Rapamycin Complex 2 - mTORC2) Akt-тық негізгі реттегіші екендігін анықтаған болатынбыз. Осы жаңа кешен mTORC2-нің бұл маңызды қызметі ісікті зерттеу жұмыстары үшін үлкен қызығушылық тудырып отыр. Берілген жұмыстың орталық гипотезасы - клеткалық трансформация кезінде mTORC2 активтенуі өз кезегінде сүт безі біріншілік ісігінің дамуындағы аса маңызды сатысы ретінде Akt сигналдық жолының активтелуіне себеп болады. Біздің зерттеуімізге сәйкес, mTORC2 активтілігін жанама бағалауға негізделген сүт безі қатерлі ісігінің жаңа маркерін дайындау мүмкіндігі бар. Онымен қоса, Akt гиперактивациясымен сипатталатын сүт безі ісігіне бағытталған mTORC2 спецификалық ингибиторын жасау өте пайдалы болар еді. mTORC2 реттелуін зерттеу аталған ісік түрінің терапиясы бағытындағы зерттеу жұмыстарының болашағы зор саласы болып табылады. Жаңа маркер mTORC2-тәуелді ісік түрлерін анықтап, сүт безі ісігінің ерте диагностикасы және бағалауы үшін қолданылады. mTORC2 спецификалық ингибиторын жасау сүт безі ісігі кезіндегі өсу факторлары сигналдық жолдарын мақсатты тежеуді қамтамасыз етеді.

Түйін сөздер риктор, сүт безі қатерлі ісігі, mTOR, mTORC1, mTORC2, инсулин тәуелді өсу факторы

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Фосфорилирование риктора зависит от фактора роста в клетках рака молочной железы

Аннотация: Несмотря на успехи в ранней диагностике, рак молочной железы все еще остается серьезным заболеванием, из-за которого ежегодно умирают около 40 000 женщин. Возникновение и развитие рака характеризуются потерей контроля над сигнальными путями ростовых факторов. Трансформация эпителиальных клеток молочной железы и развитие злокачественного фенотипа являются многоэтапным процессом, обусловленным высокоселективным давлением. Основным фактором в опухолеобразовании считается активация клеточных сигнальных путей, вовлеченных в регуляцию пролиферации, жизнестойкости и миграции клеток. В связи с этим одним из часто активируемых сигнальных компонентов в образовании опухоли у человека является Akt, который обеспечивает включение механизмов пролиферации и выживаемости у раковых клеток, необходимых для продолжительного роста опухоли. Ранее мы установили комплекс 2 мишени рапамицина (Target of Rapamycin Complex 2 - mTORC2) млекопитающих в качестве ключевого регулятора Akt. Эта важная роль нового комплекса mTORC2 вызывает огромный интерес в онкологии. Центральная гипотеза данной работы заключается в том, что во время клеточной трансформации как важнейший этап в развитии первичной опухоли молочной железы активируется mTORC2, что в свою очередь ведет к активации сигнального

пути Akt. Согласно нашему исследованию, является возможным разработка нового маркера рака молочной железы на основе косвенной оценки активности mTORC2. Кроме того, было бы чрезвычайно полезно создать специфический ингибитор mTORC2, направленный на рак молочный железы с гиперактивацией Akt. Изучение регуляции mTORC2 является перспективной областью исследований в целях разработки методов лечения данного типа рака. Новый маркер определит mTORC2-зависимые типы рака и будет применяться для ранней диагностики и оценки рака молочной железы. Создание специфического ингибитора mTORC2 обеспечит целенаправленное подавление сигнальных путей факторов роста при раке молочной железы.

Ключевые слова: риктор, рак молочной железы, mTOR, mTORC1, mTORC2, инсулино-подобный фактор роста 1

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