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Chapter 8

ErbB2 Receptor in Breast Cancer: Implications in Cancer Cell Migration, Invasion and Resistance to Targeted Therapy

Maria del Pilar Camacho-Leal,
Marianna Sciortino and Sara Cabodi

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Abstract

Overexpression of ErbB2 is found in several types of human carcinomas. In breast tumors, ErbB2 overexpression is detected in up to 20% of patients. Breast cancers in with amplification of ErbB2 are characterized by rapid tumor growth, lower survival rate and increased disease progression. The molecular mechanisms underlying the oncogenic action of ErbB2 involve a complex signaling network that tightly regulates malignant cell migration and invasion and hence metastatic potential. Recent efforts have been made to identify gene expression signatures of ErbB2-positive invasive breast cancers that may represent important mediators of ErbB2-induced tumorigenesis and metastatic progression.

In this chapter, we will discuss the canonical ErbB2 signaling pathways responsible for tumor growth and dissemination along with newly identified mediators such as adaptor protein p130Cas and miRNAs. From a therapeutic point of view, the treatment with anti-ErbB2 monoclonal antibody trastuzumab has greatly improved the outcomes of patients with ErbB2 aggressive cancer. Nevertheless, de novo and acquired resistance to trastuzumab therapy still represent a major clinical problem. In the second part of the chapter, we will provide an overview of the mechanisms so far implicated in the onset of resistance to targeted therapy and of the new strategies to overcome resistance.

Keywords: ErbB2, breast cancer, molecular mechanisms, treatment
1. Introduction

Breast cancer is the leading cause of cancer-related death in women worldwide [1]. Despite significant advances in breast cancer diagnosis and treatment, several major unresolved clinical and scientific problems still remain, such as the understanding of the causes of tumor progression and resistance and how to predict them.

ErbB2 is a well-known oncoprotein that belongs to the epidermal growth factor receptor (EGFR) family. It is overexpressed approximately in 20% of invasive breast cancers [2]. In particular, overexpression of ErbB2 has been demonstrated to promote breast cancer invasion and metastasis and to correlate with poor patient survival [3–6]. ErbB2 is also overexpressed in noninvasive mammary ductal carcinomas in situ (DCIS) [7]. Indeed, ErbB2 amplification or overexpression seems to be crucial but not sufficient for the transition from in situ to invasive cancer and additional hits are required for the progression of ErbB2-positive tumors. Although the molecular and genetic events underlying ErbB2-positive tumor invasion and metastasis are still not fully understood, intense investigation has led to the notion that molecules involved in cell adhesion and migration are critical in this process [8].

The identification of the deregulated ErbB2 pathway in breast cancer pathogenesis has led to the development of ErbB2-targeted therapies. Although ErbB2 overexpression identifies patients who are likely to respond to therapy with trastuzumab, not all patients benefit from treatment. To date, approximately 15% of patients relapse after therapy due to de novo or acquired resistance, thus it is of extreme importance to better understand the factors that contribute to therapy resistance of ErbB2-positive breast cancer tumors in order to identify novel therapeutic strategies to overcome resistance [9–11].

2. Molecular mechanisms of ErbB2 activation

Downstream signaling pathways are activated upon ErbB2 receptor activation through either heterodimerization with ligand bound EGFR, ErbB3, or ErbB4 family receptors, or in presence of overexpression of ErbB2 due to gene amplification, by ligand independent homodimerization [12]. The homo/heterodimerization promotes the receptor activation that in turn leads to tyrosine phosphorylation of the C-terminal residues. Numerous phosphorylation sites exist within the cytoplasmic domain of ErbB2, these sites are essential for protein-protein interactions and induction of the signaling cascades downstream to ErbB2 receptor activation. To this regard, the activation of the phosphoinositide 3-kinase (PI3K) and Ras/RAF/MEK/ERK1/2 pathways are hallmarks of ErbB2 activation.

Besides the canonical interaction with the member of the ErbB family, it has been recently demonstrated that activation of ErbB2 can be induced through its interaction with additional transmembrane partners. Among them Mucin 1 that is overexpressed in breast cancer and has been shown to interact with EGFR and ErbB2 leading to activation of PI3K and MAPKs pathways [13]. In addition, it has been demonstrated that leptin receptor upon leptin binding can phos-
phorylate and activate ErbB2 contributing to activation of mitogen-activated protein kinase 1 (MAPK) activity [14]. It is worth noting that further amplification of the ErbB2 signaling may derive from its crosstalk with other signaling mediators. For instance, it has been demonstrated that ErbB2 can cross-talk with hormone receptors, insulin growth factor receptor (IGFR), protein phosphatases, transforming growth beta receptor (TGFR-beta) and ion channels resulting in a complex signaling network that contribute to tumor growth and progression [15].

2.1. Canonical ErbB2 signaling network

Several downstream signaling pathways are activated after ErbB2 receptor activation leading to the regulation of cell proliferation, growth and survival as well as invasion and angiogenesis [15]. Tyrosine residues phosphorylation resulting from receptor activation can recruit a variety of intracellular adaptor and scaffold proteins that in turn mediate the activation of downstream signaling pathways.

One of the most important pathways activated by ErbB2 signaling is the RAS-MAPK pathway [16]. The activation of the MAPK pathway controls cell proliferation, survival and migration and alteration of this pathway have been linked with many diseases including cancer.

Upon ErbB2 activation, the adaptor molecule growth factor receptor-bound protein 2 (GRB2) binds through its SH2 domain to the phosphorylated intracellular tail of ErbB2. GRB2 bound to the receptor recruits the adaptor protein son of sevenless (SOS) determining its activation. Active SOS can trigger the activation of RAS by inducing the transition the GDP-inactive to the GTP-active state. The activation of RAS promotes a cascade of downstream kinase activation that ultimately leads to the phosphorylation and activation of extracellular signal-regulated kinases 1 and 2 (ERK1, ERK2) [17, 18]. Activated ERK proteins phosphorylate a number of transcription factors such as Elk-1, c-Fos and c-Jun among others, that regulate the expression of genes implicated in cell growth, differentiation, proliferation, survival and migration [15, 19].

The PI3K/AKT is the second canonic pathway activated by ErbB2 and due to its relevance in cell proliferation, survival, protein synthesis, invasion and drug resistance has received much attention to develop anticancer targeted therapy [17, 18, 20]. Upon receptor activation, the p85 subunit of PI3K binds to tyrosine-phosphorylated residues of ErbB2. This recruitment determines the release of the 110 subunit of PI3K and allows the formation of PI3K heterodimers that can phosphorylate PIP2 substrate in PIP3 [21]. Ultimately PIP3 promotes the localization of AKT at cell membrane and its phosphorylation by PDK1 and mTOR complex 2. AKT represents the major effector of the PI3K/AKT signaling pathway leading to the regulation of many cell functions such as cell survival, cell growth and proliferation [21] (Figure 1).

2.2. ErbB2 signaling mediated by the adaptor proteins p130Cas

It is clear that activation of canonical ErbB2 signaling can be achieved through the recruitment of signaling proteins to the receptor. It is now emerging that p130Cas adaptor protein can mediate the activation of ErbB2 downstream signaling pathways. p130Cas/BCAR1 scaffold molecule is a signaling molecule involved in the linkage of actin cytoskeleton to the extracellular matrix during cell migration, cell invasion and cell transformation [22, 23] and it has
been described as an essential transducer element in ErbB2 transformation and progression [24]. Due to its modular structure, p130Cas has been described to play a crucial role in signaling originating from many amplified or mutated oncogenes, by undergoing hyperphosphorylation and association with multiple signaling partners required for transformation [22].

It was recently demonstrated that overexpression of p130Cas in ErbB2 breast cancers correlates with poor survival and increased progression. In particular, p130Cas is required for ErbB2-dependent transformation and invasion both \textit{in vitro} and \textit{in vivo} models. Indeed, silencing of p130Cas is sufficient to inhibit ErbB2 orthotopic tumor growth in mice. The administration of p130Cas stabilized siRNAs by intranipple injection in the mammary glands of mice with spontaneous ErbB2 cancer lesions, significantly impaired lesions growth, indicating that p130Cas might be a potential therapeutic target [24]. It has also been reported that p130Cas binds to ErbB2 and its overexpression is sufficient to transactivate the ErbB2 receptor leading to the formation of a macromolecular signaling complex, in which Src and

\textbf{Figure 1.} Canonical ErbB2 signaling network. ErbB2 activation leads to the activation of the phosphoinositide-3-kinase (PI3K)/AKT and the mitogen-activated protein kinase (RAS/RAF/MAPK) pathways that trigger cell proliferation, growth and survival.
p125Fak kinases are present, that sustains ErbB2 downstream signaling pathways leading to activation of both MAPK and PI3K pathways regulating cell transformation, invasion and migration [24, 25]. Interestingly, concomitant p130Cas/ErbB2 overexpression accelerates the onset of mammary tumors, which are characterized at the molecular level by increased activation of c-Src and Akt [26]. Notably, a positive correlation between the expression of BCAR1/p130Cas and ErbB2 has been found in human breast cancers and the coexpression of these two genes is associated with shorter overall survival and a higher risk of developing distant metastasis [25, 26] (Figure 2).

2.3. MicroRNA in ErbB2-overexpressing cancer

The discovery of microRNAs (miRNAs) has provided new perspectives to study cancer at the molecular level. These noncoding regulatory RNA molecules of ~22 nucleotides have emerged as important cancer biomarkers, effectors and targets. Alteration of miRNAs expression has been correlated with a variety of human diseases, including breast cancer [27].
It was initially demonstrated that the overexpression of miR-125a and miR-125b in human breast cancer cell line SKBR3 overexpressing ErbB2 was sufficient to lower ErbB2 and ErbB3 mRNA and protein levels, with consequent inhibition of anchorage-dependent growth, migration and invasion. Consistently, activation of canonical ErbB2 downstream signaling such as MAPK and PI3K/Akt pathways was severely impaired [28]. Two subsequent studies identified by using different methodologies two miRNA signatures of ErbB2 positive breast cancer. In particular, miR-520d, miR-181c, miR-302c, miR-376b, miR-30e were identified as miRNA associated with HER2 status to be added to the previously found let-7i, let-7g, miR-107, mir-10b, miR-126, miR-154 and miR-195 as miRNA characterizing HER2 status [29, 30].

These data highlight the relevance of microRNA signatures as novel breast cancer biomarkers. The consequences of the association of ErbB2 and miRNAs are still under investigation but three possible scenarios can be identified. The first one envisages the regulation of miRNAs as a consequence of ErbB2 activation. The second possibility is that miRNAs contributes to the activation of ErbB2 and to its capacity to trigger downstream-signaling pathways. The last option is that miRNAs can interfere with the response to ErbB2 targeted-therapy thereby mediating the onset of resistance.

Further investigations are needed to identify which is the crucial miRNAs implicating in the different responses to ErbB2 activation and to develop new selective anticancer therapy.

3. Role of Erbb2 in breast cancer invasion and metastasis

From the physiological point of view, ErbB2 represents an important molecule implicated in the regulation of cell proliferation, differentiation, survival and migration during embryonic development and in adults, during tissue maintenance. Importantly, during pathological conditions, ErbB2 aberrant expression and activation in breast cancer have been extensively linked to invasive, aggressive phenotype and poor prognosis [31]. Acquisition of migratory properties allow cancer cell to invade the surrounding tissues and reach the blood vessels to generate metastasis. At the cellular level, the transition from noninvasive to invasive status is characterized by loss of the epithelial characteristics such as expression of cytokeratins and E-cadherin and gain of mesenchymal traits like vimentin, fibronectin and N-cadherin through a process that is known as epithelial to mesenchymal transition (EMT) [32]. EMT promotes cancer progression by allowing cancer cells to acquire invasive properties, to metastasize and also to acquire stem cell properties [33, 34]. Interestingly, these cells that have acquired stem cell properties are characterized by increased expression of EMT genes, such as FoxC2, Zeb and N-cadherin [35, 36]. Moreover, it has been demonstrated that ErbB2 overexpression in breast cancer cell lines can enhance the stem cell population which is responsible for breast cancer progression [37].

3.1. Erbb2 invasive signaling signature

For the past years, extensive investigations have been performed in order to understand the precise mechanisms implicated in the regulation of cell invasion and metastasis as the result of ErbB2 activation. Several *in vitro* studies have pointed out the requirement of additional...
molecular hits in order to induce malignant transformation mediated by ErbB2 overexpression. For example, in nontransformed MCF10A breast epithelial three-dimensional cell cultures, ErbB2-mediated cell transformation occurs upon the activation of the TGFβ signaling [38]. Additional studies in 3D MCF10A cultures have led to the identification of signaling proteins already implicated in cytoskeletal organization and cancer cell invasion. In particular, these studies suggest that p21-activated protein kinase (PAK) family of serine/threonine kinases that function as effectors of Cdc42 and Rac, by activating the Raf/Mek/Erk and Akt pathways, cooperates with ErbB2 in transforming mammary epithelial cells [39]. More recently, using the same in vitro cell model, it was shown that the ErbB2-driven invasive phenotype requires both cathepsins B and L. Cathepsins B and L are lysosomal cysteine cathepsins that upon secretion to the extracellular space can cleave and activate urokinase plasminogen activator, heparanase and various matrix metalloproteases as well as E-cadherin and, thus, contribute to invasion and metastasis [40]. In MCF10A cells engineered to express a chimeric form of ErbB2 that can be induced to dimerize by treatment with a synthetic ligand [41], it was reported that the adaptor molecule p130Cas controls ErbB2-dependent invasion. Indeed, the overexpression of p130Cas in ErbB2-transformed mammary acini leads to activation of PI3K/Akt and Erk1/2 MAPK signaling pathways and promote invasion of mammary acini. It was further demonstrated that Erk1/2 MAPK and PI3K/Akt-signaling triggers invasion through distinct downstream effectors involving mTOR/p70S6K and Rac1 activation [25]. The relevance of p130Cas in ErbB2-dependent invasion was further assessed by identifying the coding and noncoding genes that are differentially expressed in p130Cas overexpressing and ErbB2 transformed invasive acini compared to ErbB2 transformed noninvasive multicellular structures [42].

The study of the consequences ErbB2/Neu activation in in vivo mouse models has shown that overexpression of ErbB2 seems to be enough for the induction of metastatic mammary cancer

![Diagram](image_url)

**Figure 3.** ErbB2 invasive signaling signature. ErbB2 activation impacts on EMT and cell invasion through the activation of a variety of downstream effectors.
However, there are several data demonstrating that ErbB2 cooperation with additional signaling effectors is crucial for cell transformation and invasion [22]. More recently, in vivo studies combining ErbB2/Neu with overexpression or knockout of different genes have led to the identification of several molecular targets that contribute to ErbB2-induced metastasis. These include molecules such as protein tyrosine phosphatase 1B (PTP1B), tensing homolog (PTEN), vascular adapter protein (VEGF), Gab2, EphA2 receptor tyrosine kinase, Rho GTPase activating protein p190B, receptor activator of nuclear factor-κB (RANK), estrogen receptor α, semaphorin receptor plexin-B1 and Rac-specific guanine nucleotide exchange factor DOCK1. Altogether these studies reflect the complexity of the molecular mechanisms implicated in the regulation of invasion and metastasis by ErbB2 [31] (Figure 3).


The assessment that ErbB2 overexpression correlates with aggressive breast cancer and poor survival has led to the development of targeted therapies to inhibit the receptor. Among them, the monoclonal antibody is trastuzumab and pertuzumab and the tyrosine kinase inhibitor is lapatinib [44]. Although ErbB2 overexpression identifies patients who are likely to respond to targeted therapy, not all of them benefit from the treatment. Indeed, many patients relapse after therapy due to the acquisition of primary or acquired resistance. Primary resistance might occur because of lack of target dependency or activation of compensatory pathways [45, 46]. Acquired resistance, which develops in most patients with advanced disease, may be due to the loss of the expression of the target because of continuous therapy, or to additional mutations that occur either in the target or on downstream signaling pathways that ultimately result in enhanced cell proliferation [47].

Many factors can contribute to resistance to ErbB2-targeted therapies. Among them, loss of ErbB2 amplification, compensatory mechanisms such as ErbB3 activation or the presence of p95ErbB2, a fragment of ErbB2 that cannot bind to trastuzumab as it lacks the extracellular part but can still activate the downstream pathways by retaining the ability to associate with ErbB2-signaling partners [44]. Additional factors that might be responsible for resistance to ErbB2-targeted therapies include aberrant activation of downstream signaling pathways due to mutations occurring during therapy, for example in the PI3K pathway [48, 49] or the activation of crosstalk with other receptor tyrosine kinases leading to compensatory mechanisms [50, 51].

In addition, poor internalization of ErbB2 resulting in a long half-life at the plasma membrane has been described as an important mechanism implicated in ErbB2 therapy resistance. In this context, it has been shown that Hsp90 inhibition can induce ErbB2 ubiquitination followed by its downregulation [52], however the mechanisms underlying ErbB2 down-regulation are still obscure. Recently it has been demonstrated that molecular association between p130Cas and ErbB2 protects the receptor from degradation through autophagy [53]. On this regard, increasing evidence points out that ubiquitination is an important mechanism driving autophagic degradation. Interestingly, in breast cancer cells overexpressing ErbB2, p130Cas protects ErbB2 from autophagy-mediated degradation by interfering with its ubiquitination, thus suggesting that high levels of p130Cas expression might be crucial to promote resistance to trastuzumab treatment by protecting ErbB2 from degradation [53].
In conclusion, the unraveling of the molecular mechanisms responsible for resistance would greatly contribute to improve prognosis and outcomes for patients with ErbB2 tumors allowing a better selection of patients who are likely to respond to ErbB2-targeted therapies. Moreover, the dissection of the molecular pathways might reveal new insights for the development of strategies to overcome resistance.

4.1. Overcoming resistance to targeted therapy

Two main strategies have been adopted to try to overcome resistance to trastuzumab therapy. One strategy is still based on targeting ErbB2 either by maintaining trastuzumab therapy beyond progression, since it has been demonstrated that some patients could benefit of trastuzumab therapy with progressive disease [54, 55], or by treatment with TKI inhibitor lapatinib in combination with chemotherapy [56]. Another option is to treat trastuzumab resistant patients with the T-DM1. T-DM1 consists of an antibody (trastuzumab) conjugated with a microtubule inhibitor (maytansine derivative) with cytotoxic activity (developed by Genentech, Inc.). At present, many new drugs targeting ErbB2 are undergoing clinical investigation in patients with ErbB2-resistant breast cancer overexpression. Since resistance to ErbB2-targeted therapy might occur as a result of aberrant activation of signaling pathways downstream to the receptor, the other strategy adopted to overcome resistance to trastuzumab is to target downstream signaling pathways known to be activated by ErbB2.

A major effort has been undertaken to inhibit the PI3K/Akt/mTOR pathway that, as mentioned before, is one of the most relevant downstream signaling activated by ErbB2. Indeed, alterations of PI3K/Akt pathway result in the upregulation of the mTOR pathway that in turn promotes translation and increased cellular proliferation [57, 58]. These signaling events have been characterized in breast cancer models in which the PI3K/Akt/mTOR axis is constitutively activated and responsible for the acquirement of resistance to ErbB2-targeted therapy [59]. It has been also described that the deregulation of this pathway accounts for gain of function mutations \textit{PIK3CA} gene and/or mutations in AKT1, amplification of AKT2 and loss of PTEN [60]. The correlation between PTEN loss and trastuzumab and lapatinib resistance has also been reported [49, 61]. \textit{PIK3CA} gene mutations acquired during disease progression are likely to reflect increased activation of the PI3K pathway and therefore suggest possible implications in resistance [47]. Consistently with this hypothesis, in vitro data show that ErbB2 gene amplification and \textit{PI3KCA} gene mutations are associated with resistance to ErbB2-targeted agents [49, 62, 63] and PTEN loss or \textit{PIK3CA} gene mutations have been linked to resistance to ErbB2 targeted therapy [48]. Since the serine/threonine kinase mTOR represents the final sensor of the ErbB2-dependent activation of the PI3K/AKT pathway and it is negatively regulated by PTEN, it is conceivable that targeting mTOR might be more efficacious than targeting multiple pathways with different strategies [48, 64] to interfere with tumor progression and to prevent resistance to ErbB2-targeted therapy. Consequently, several inhibitors of mTOR have been developed and tested in \textit{in vitro} and \textit{in vivo} models of trastuzumab resistance showing that the combined therapy (trastuzumab + mTOR inhibitor) was efficacious in inhibiting tumor
growth [65]. The mTOR inhibitor everolimus is currently being tested in combination with trastuzumab and with different chemotherapeutic drugs in clinical studies to evaluate its potential in overcoming resistance to ErbB2-targeted therapy [66–68]. Besides inhibitors of the PI3K/Akt/mTOR pathways, additional inhibitors against other pathways or molecules known to play a role in ErbB2-resistance to targeted therapy have been developed. Among them IGFR, Hsp90, VEGF and telomerase inhibitors whose mechanisms of action and ongoing preclinical and clinical studies have been reviewed in [69].

Further ongoing characterization of the key effectors implicated in the resistance of ErbB2-targeted therapy might provide new efficacious pharmaceutics to improve or to overcome trastuzumab resistance.

5. Conclusion

Many progresses have been made in the understanding of the molecular mechanisms leading to the activation of ErbB2 and its downstream signaling pathways. Further studies are needed for a better comprehension of the mechanisms that lead to resistance to ErbB2-targeted treatment and especially to identify the crucial molecules deserving a therapeutic approach. New efforts have to be undertaken to see whether new modulators of ErbB2 such as miRNAs and adaptor proteins like p130Cas can be used as new therapeutic targets.

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Abbreviations

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<tr>
<th>Abbreviation</th>
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<tr>
<td>AKT</td>
<td>protein kinase B (PKB)</td>
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<td>DCIS</td>
<td>ductal carcinomas in situ</td>
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<tr>
<td>EGF</td>
<td>epidermal growth factor receptor</td>
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<tr>
<td>EphA2</td>
<td>EPH receptor A2</td>
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<tr>
<td>ERK1/2</td>
<td>extracellular signal–regulated kinase 1/2</td>
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<td>EMT</td>
<td>epithelial–mesenchymal transition</td>
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<td>FAK</td>
<td>focal adhesion kinase</td>
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<td>FoxC2</td>
<td>forkhead box protein C2</td>
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<tr>
<td>Gab2</td>
<td>GRB2-associated-binding protein 2</td>
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GRB2  growth factor receptor-bound protein 2
IGFR  insulin growth factor receptor
MAPK  mitogen-activated protein kinase 1
mTOR  mammalian target of rapamycin complex 1
PI3K  phosphoinositide 3-kinase
PTP1B  protein tyrosine phosphatase 1B
PTEN  phosphatase and tensin homolog
PLXNB1  plexin-B1
RANK  receptor activator of nuclear factor-κB
Rac  Ras-related C3 botulinum toxin substrate 1RAF
Rho  Ras homolog gene family, member A
Src  proto-oncogene tyrosine-protein kinase
SOS  adaptor protein son of sevenless
TGFbeta  transforming growth factor beta
TGFR-beta  transforming growth factor beta receptor
VEGF  vascular endothelial growth factor
ZEB1  zinc finger E-box-binding homeobox 1

Author details

Maria del Pilar Camacho-Leal*, Marianna Sciortino and Sara Cabodi
*Address all correspondence to: mariadelpilar.camacholeal@unito.it
Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center (MBC), University of Turin, Turin, Italy

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