Expression of the Insulin Receptor Substrate 1 in primary tumors and lymph node metastases in breast cancer: correlations with Bcl-xL and Bax proteins

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In our previous investigation Insulin Receptor Substrate 1 (IRS-1) correlated with proliferation marker Ki-67 in human breast cancer. The aim of the present study was to assess relationships between IRS-1 expression and antiapoptotic Bcl-xL as well as proapoptotic Bax proteins, assessed by immunohistochemistry, in primary tumors and lymph node metastases of breast cancer. IRS-1 is positively associated with both Bcl-xL and Bax in primary and metastatic tumors. Thus, our results could suggest that IRS-1 might affect turnover of cancer cells and breast cancer progression through activation of mitogenesis and participation in the regulation of the balance between anti- and proapoptotic pathways.

Key words: insulin receptor substrate 1, Bax, Bcl-xL, breast cancer

The Insulin Receptor Substrate 1 (IRS-1) is responsible for connecting insulin-like growth factor-I receptor (IGF-IR) activation with different cellular signaling pathways [1, 10, 13]. Phosphorylated IRS-1 is responsible for binding and activation of signaling proteins implicated in mitogenesis and protection from apoptosis, such as p85 subunit of phosphatidylinositol 3-kinase (PI3-K) as well as the Mitogen Activated Protein (MAP) kinase pathway [6, 7]. Disturbances in IRS-1 expression have been associated with pathogenesis of different malignancies such as breast, pancreas, prostate and liver cancers [2, 9, 10]. Despite the well known role of IGF-I, IGF-IR and IRS-1 in inhibition of apoptosis, in recent years it has been suggested that these proteins could also enhance apoptosis, but exact mechanisms of their actions are still unclear [3, 8, 14, 15]. Therefore, our goal was to compare IRS-1 with antiapoptotic Bcl-xL and proapoptotic Bax with focus on their tissue expressions in primary tumors as well as in lymph node metastases of breast cancer.

Material and methods

The study comprised 109 women (mean 54.4 years) having total mastectomy for primary invasive breast cancer, ductal type: 63/109 tumors in G2 grade and 46/109 in G3 grade. 52/109 women had involved lymph nodes at the time of diagnosis.

Immunohistochemical studies were performed in sections from primary and metastatic tumors, as described previously [4], using antibodies: rabbit polyclonal IRS-1 (Santa Cruz Biotechnology, [SCBt], USA) at a 1:100 dilution; goat polyclonal Bcl-xL (SCBt, USA) at a 1:300 dilution and goat polyclonal Bax (SCBt, USA) at a 1:100 dilution. In negative controls the primary antibodies were omitted.

The expression of each marker was analyzed by light microscopy in 10 different tumor fields and the mean percentage of tumor cells displaying positive staining was scored. The immunoreactivity of IRS-1 was classified as follows: 0, <10% positive cells; 1+, 10–50% positive cells with weak staining; 2+, 10–50% positive cells with strong staining or >50% positive cells with weak staining; 3+, >50% positive cells with strong staining. Bcl-xL and Bax were classified as follows: 0, <10% positive cells; 1+, 10–50% positive cells; 2+, >50% positive cells.

Analysis of correlations between the expression of IRS-1, Bax, Bcl-xL in the primary tumors and in the lymph nodes was performed using the Spearman test. Probabilities of $p<0.05$ were assumed as statistically significant.

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Results

IRS-1 and apoptotic markers (Bax and Bcl-xL) in primary breast cancers. Immunohistochemical analysis revealed positive, cytoplasmatic IRS-1, Bax as well as Bcl-xL microgranular staining in 69.7%, 57.9% and 78% of cases, respectively. The expression of IRS-1, Bax and Bcl-xL was undetectable in control samples, where immunodetection was performed with the omission of the primary antibodies.

In the primary tumor, we found a significant positive correlation between IRS-1 and Bax expression ($p<0.001$, $r=0.346$) as well as between IRS-1 and Bcl-xL ($p=0.001$, $r=0.315$; Tab. 1). Different relationships between studied proteins depended on tumor grade and lymph node status (Tab. 1).

IRS-1 and apoptotic markers (Bax and Bcl-xL) in metastases to lymph nodes. The positive expression of IRS-1, Bax and Bcl-xL was found in 76.2%, 85.7% and 92.8% of metastatic tumors, respectively. In the whole group of breast cancer patients we found positive correlation between IRS-1 and Bax expression in lymph node metastases ($p=0.037$, $r=0.356$) as well as between IRS-1 and Bcl-xL ($p=0.004$, $r=0.447$). In metastatic tumors, derived from G3, but not from G2 primary tumors, we noted relationships between IRS-1 and Bcl-xL as well as Bax (Tab. 2).

Table 1. Analysis of relationships between IRS-1 and Bcl-xL as well as Bax expression in primary tumors of the breast cancer

Primary tumors	Compared markers		r	p
All primary tumors; n=109	IRS-1	Bax	0.346	<0.0001
	IRS-1	Bcl-xL	0.315	0.001
Moderately differentiated tumors (G2); n=63	IRS-1	Bax	0.249	0.075
	IRS-1	Bcl-xL	0.342	0.010
Poorly differentiated tumors (G3); n=46	IRS-1	Bax	0.278	0.064
	IRS-1	Bcl-xL	0.209	N.S.
Tumors with involved lymph nodes (N+); n=52	IRS-1	Bax	0.409	0.007
	IRS-1	Bcl-xL	0.331	0.026
Tumors without involved lymph nodes (N-); n=57	IRS-1	Bax	0.335	0.021
	IRS-1	Bcl-xL	0.312	0.029

N.S. – not significance; n – number of cases

Table 2. Analysis of relationships between IRS-1 and Bcl-xL as well as Bax expression in metastases to lymph nodes

Metastases to lymph nodes	Compared markers		r	p
All studied lymph node metastases; n=40	IRS-1	Bax	0.356	0.037
	IRS-1	Bcl-xL	0.447	0.004
Metastases derived from primary tumors in G2 grade; n=21	IRS-1	Bax	0.132	N.S.
	IRS-1	Bcl-xL	0.097	N.S.
Metastases derived from primary tumors in G3 grade; n=19	IRS-1	Bax	0.723	0.002
	IRS-1	Bcl-xL	0.757	<0.0001

N.S. – not significance, n – number of cases

Discussion

Members of the Insulin-like Growth Factor (IGF) system play an important role in the regulation of mitogenesis, transformation and apoptosis of different cancer cells [1, 4, 5, 7, 10]. Insulin Receptor Substrate-1 (IRS-1) was reported to show an antiapoptotic activity in several studies. For instance, TANAKA and WANDS [11] suspected that IRS-I-mediated signals may prevent programmed cell death as survival factors for hepatocellular carcinoma. According to UENO et al [12] an antiapoptotic activity of IRS-1 was *in vitro* connected with complexes of IRS-1 with antiapoptotic Bcl-2 and Bcl-xL. IRS-1 bound to the loop domains of antiapoptotic proteins and by means of that regulated their phosphorylation. It is thought that dephosphorylation amplifies the function of antiapoptotic proteins, so in UENO's et al [12] opinion the IRS-1 mediated signals enhance the activity of serine/threonine phosphatases. On the other hand, lack of loop domains made Bax and Bik incapable of binding with IRS-1. Our positive correlations that link IRS-1 with anti- as well as proapoptotic proteins could result in contradictory conclusions. Anyway, it should be underlined that we did not try to explain functional relationships between studied proteins. We found only some associations of statistical significance that occurred when comparing tissue levels of mentioned factors. In our opinion our data are important starting point to inquire a real nature of interactions between these proteins. Nevertheless, revealed positive correlations indicate a great probability of functional dependence among IRS-1, Bax and Bcl-xL. It is also possible that influence of IRS-1 on apoptosis can be diverse. It has recently been uncovered that IGF-I, IGF-IR and IRS-1 could also play a role in proapoptotic pathways besides protection from apoptosis [3, 8, 14, 15]. WIEDMANN et al [14] found that proapoptotic Fas signaling system was associated with significantly increased levels of Fas receptor in mouse livers with IRS-1 overexpression. Biologically active Fas receptor was manifested by the higher grades of hepatocellular apoptosis. WIEDMANN et al [14] suggested that the major mechanism compensating for increased proliferation in cells with overexpressed IRS-1 could be increased sensitivity to apoptosis. Additionally, FEDERICI et al [3] noticed that polymorphism in IRS-1 (Gly to Arg change at codon 972; Arg^{972}-IRS-1) was associated with

apoptosis of pancreatic islets. Arg^{972}-IRS-1 exhibited impaired IRS-1-associated phosphatidylinositol 3-kinase (PI3-K) and Akt activity and increased apoptosis. Akt promotes survival by phosphorylation and the subsequent inactivation of components of the cell death machinery. Among these is BAD, which exerts its proapoptotic effect partly by sequestering Bcl-xL. Upon phosphorylation BAD dissociated from Bcl-xL, and the released Bcl-xL then promotes cell survival. In cells expressing Arg^{972}-IRS-1, phosphorylation of BAD was reduced as compared with cells expressing wild-type IRS-1, which resulted in an increased binding to Bcl-xL and increased apoptosis [3]. Apart from induction of proliferation and cell growth, IGF-I stimulated apoptosis in human MG63 osteosarcoma cells, that was assessed by enhanced caspase-3 activation, although cell growth and survival still predominated [8]. Similarly, WU et al [15] observed increased activity of proapoptotic p38-MAPK pathway in cells expressing truncated form of IGF-IR.

Presented study for the first time demonstrates positive correlation of IRS-1 with antiapoptotic Bcl-xL as well as with proapoptotic Bax proteins in primary breast cancer as well as in lymph node metastases. Previously, in ERα-positive breast cancer, we found an association between IRS-1 and proliferation marker Ki-67 [4]. Moreover, we also noted a correlation between IRS-1 expression and poorly differentiated primary tumors as well as lymph node involvement [4]. Our present and previous results seem to agree with observations of other investigators in the topic of anti- and proapoptotic activity of IRS-1. Obviously they all indicate that enhanced breast cancer growth and proliferation, could be counteracted by the stimulation of apoptosis. At this step of knowledge, both cell survival and apoptosis are associated with IRS-1 overexpression. It would be not so strange if we consider IRS-1 as a signaling molecule, which overexpression in cancer cells could activate numerous intracellular pathways. In our opinion it is quite possible, that IRS-1, which involve proliferative and antiapoptotic responses, could also stimulate proapoptotic mechanisms. Consequently, IRS-1 through activation of mitogenesis and participation in the regulation of the balance between anti- and proapoptotic pathways might affect tumor cell turnover and breast cancer progression. However, it is a very important future task to evaluate the functional relationship between IRS-1 and Bcl-xL and Bax proteins as well as the prognostic implications of increased IRS-1 as well as Bcl-xL and Bax expression in lymph node metastases.

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