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THE EFFECT OF SYK ON BREAST CANCER PROGRESSION

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ABSTRACT

Spleen tyrosine kinase (Syk) is a protein tyrosine kinase that has long been known to various roles in cells of haemopoietic origin. Discovery of Syk in cells of non-haemopoietic origin such as epithelial cells, hepatocytes, fibroblasts, and neuronal cells has generated considerable interest over their non immunogenic functions in these cells. The presence of Syk in normal human breast tissue, benign breast lesions and low-tumorigenic breast cancer cell lines along with the fact that its expression is considerably decreased in cell lines of higher invasive nature has put Syk as a novel emerging target. This review shall look briefly into the structure and function of Syk and provide a detailed overview of the various research that has been carried out on the role of Syk in breast cancer and the results obtained from these studies.

KEYWORDS: Syk, Prevention of metastasis, Breast cancer.

INTRODUCTION

Breast cancer is turning out to be a major menace for the entire women populace of the world. It is said to be the leading cause of cancer mortality in women.[1] Due to advances in treatment there has been a significant decline in mortality.[2] This decline can be attributed to discovery of various specific target proteins and receptors that are over expressed or modified in some way in breast cancer. One such potential protein that has caught the fancy of researchers is Syk.

Syk was initially discovered by Zioncheck et al. when they extracted and isolated a protein from bovine thymus which they called as p40 kinase. They reported that the presence of a p40 kinase containing a ATP binding site and stated it to be competent of intramolecular
autophosphorylation of tyrosine residues. Further experimentation by the same group revealed that the antibodies that were prepared for p40 cross reacted with a 72-kDa protein-tyrosine kinase (p72) that is found in spleen, thymus and B and T cells. Now it is a well known fact that the p40 fragment discovered was the catalytic site of the p72 protein kinase which we know today by the name of Syk (Spleen tyrosine kinase).

**STRUCTURE OF SYK**

Syk is a protein tyrosine kinase that has been known to play a role of significance in signal transduction pathway of immune cells. It consists of a C-terminal kinase domain, an tandem N-terminal SH2 domain and two ‘linker’ region which are designated as interdomain A (present between N-SH2 and C-SH2 domains) and interdomain B (present between C-SH2 and kinase domain). The SH2 domain serves to bind a diphosphorylated ITAM (immunoreceptor tyrosine-based activation motif). The linker regions helps in enlisting various signalling molecules that play an active role in downstream signalling of events and in proper alignment of the SH2 region to bind with ITAM. Figure 1 shows a schematic representation of Syk structure.

![Schematic representation of Syk](image)

Figure 1: Schematic representation of Syk

Syk has been known to play a role in signal transduction of B-cell receptor, Fc receptors and activation of NK cells. The process of activation occurs such that ITAMs are phosphorylated, Syk then translocates to this phosphorylated ITAM by interaction of the SH2 domain, which leads to conformational changes in Syk. The activated Syk then causes activation of various molecules and proteins such as Vav family of guanine nucleotide exchange factors, BLNK(B-cell linker protein) etc. The presence of Syk in cells of non haemopoetic origin has created a furor in the research community as till then Syk was only seen only as a kinase involved in immune cell development and differentiation. This finding opened up new avenues for prediction of working of Syk in various non haemopoetic tissues.
ROLE OF SYK IN BREAST CANCER

The discovery of Syk in normal and benign breast tissue by Coopman et al. created a furore in the research community as they implicated Syk as a negative tumor modulator in breast cancer, till that time tyrosine kinases were largely seen as positive tumour modulators. Syk itself fits into this category, as it has shown to have positive role in B and T cell lymphoma.\textsuperscript{[14,15]} The paradoxical role of Syk is observed when it is found to prevent metastasis in gastric cancer and prevent the spread of melanoma yet act as a tumour promoter in lymphoma.\textsuperscript{[16,17,15]} The information of role of Syk in suppression of other type of cancers is very minute when compared with that of breast cancer.

The tumour suppressor role of Syk came to light when Coopman et al. in their research observed that in group of well defined cell lines taken for study, the cell lines that were of low invasive nature expressed high levels of Syk and cell lines that were of high invasive nature expressed low levels of Syk. They further reported that by transfecting wild type Syk into a predominantly Syk negative cell line and injecting the pooled clones into mammary pads of athymic nude mice, the tumor that was formed had a fivefold lesser mean tumor volume as compared with that of the negative control. Injection of the Syk transfected cells in tail vein of mice was done to observe formation of metastatic colonies in their lung, it was seen that only one of the animals with Syk transfected cells showed formation of a single metastatic colony whereas all the animals of the negative control showed formation of multiple colonies. This goes on to prove that Syk acts as an negative modulator for breast cancer and also gives considerable evidence to the fact that Syk can retard growth and malignancy in breast tumors.\textsuperscript{[18]}

Based on the above research studies have been done to document the progressive loss of Syk with increase of invasiveness of breast cancer from normal to ductal to invasive.\textsuperscript{[19]} It has also been suggested to use this progressive loss of Syk as a diagnostic marker for progression of breast cancer.\textsuperscript{[20,21]}

Role of Syk in Prevention Of Mammary Cells Metastasis

The discovery of Syk in breast tissue coupled with the fact that cells transfected with Syk were unable to metastasize, even in highly invasive forms of breast cancer cell lines. This phenomenon prompted the researchers to ask the question whether Syk played any role in suppressing cell motility which had hitherto shown to be an important factor for metastasis.
Intrigued by this possibility, Mahableshwar and Kundu decided to evaluate the role of Syk in suppression of cell motility. Cell migration and extracellular matrix invasion are known means of cancer metastasis.\(^{[22, 23]}\) Urokinase-type plasminogen activator (uPA) converts plasminogen to plasmin which helps in cell invasion by degradation of cellular proteins.\(^{[24]}\)

The activation of uPA by PI 3' kinase dependent activation of NF\(_k\)B(nuclear factor kappa-light-chain-enhancer of activated B cells) has been implicated in cancer metastasis. The interaction of the regulatory subunit of PI 3'kinase with tyrosine phosphorylated I\(_k\)B\(_\alpha\)(nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) leads to the activation of NF\(_k\)B.\(^{[25]}\) In their study Mahableshwar and Kundu took cells from MCF-7 (Syk expressing) and MDA-MB-231(non Syk expressing). The cells from MDA-MB-231 were transfected with wild type Syk and MCF-7 cells were transfected with Syk-specific antisense phosphorothioate oligonucleotide (ASSyk). To further prove the point that Syk plays an active role in cell suppression; a panel of wild type Syk transfected MDA-MB-231 was treated with piceatannol a Syk inhibitor. To ascertain whether PV (Pervanadate), a tyrosine phosphatase inhibitor (group of enzymes that remove phosphate groups from phosphorylated tyrosine residues), regulates PI 3'kinase dependent cell migration, cells of both MCF-7 and MDA-MB-231 were treated in presence and absence of a PI 3'-kinase inhibitor. The study resulted in findings that Syk inhibited PI 3'kinase activity in both MDA-MB-231(high invasive) and MCF-7(low invasive) breast cancer cells, furthermore Syk was able to suppress PV induced interaction of PI 3'kinase with I\(_k\)B\(_\alpha\). The presence of piceatannol in cells enhanced their NFkB activity due to inhibition of Syk by piceatannol. It was also shown that Syk inhibits uPA secretion in both MCF-7 and MDA-MB-231. Their findings show that Syk down regulates NFkB by suppressing interaction between regulatory domain of PI 3'kinase and I\(_k\)B\(_\alpha\). They also concluded that an increased level of PI 3'kinase can be taken as sign of metastasis.\(^{[26]}\)

Another means through which Syk regulates NF-kB was shown by Fei et al. as they showed the interaction between Syk and the calpain- calpastatin system. Calpain has been implicated in large number of physiological process such as regulation of gene expression, control of cell cycle, and regulation of apoptosis etc.\(^{[27]}\) Calpain- calpastatin system is composed of \(\mu\)-calpain, m-calpin and CAST (calpastatin) which is the only known endogenous inhibitor of calpain.\(^{[28]}\) Tumour invasion causes a dysregulation of the calpain system.\(^{[29]}\) Intrigued by the possibility that a reciprocal regulation may exist between Syk and calpain system Fei et al. decided to explore the role of Syk in calpain-calpastatin system. They examined the effect of
Syk on the calpain mediated proteolysis of ReIA (p65) subunit of NF-kB expressed in MCF-7 cells. It was found that Syk prevented the proteolysis of ReIA by stimulating calpastatin, which is required for the inhibition of calpain that is responsible for proteolysis of ReIA.

They concluded that Syk regulates calpain activity through upregulation of CAST, modulating the level of intracellular calcium; they further showed that calpain inhibition enhanced TNF-α-induced activation of NF-kB and also integrin ligation induced tyrosine phosphorylation which is necessary for cell survival and tumour suppression.[30]

The overall effect of Syk on NF-kB is highly varied as some workers report the upregulation of NF-kB in Syk expressing cells while other workers report the exact opposite, but it should be noted here that NF-kB has been known to have a varied and diverse role concerning its activation and effects in cancer.[31]

Working on the same lines Zahng et al. tried to establish a relationship between Syk expression and proteins required for cell adhesion and motility. It has been shown that many proteins that are responsible for cell adhesion are broken down by kinases, [32] but in contradiction to this nature of kinases Zahng et al. showed that Syk kinase shows a role in increasing cell adhesion and cell motility. They showed an interaction between Syk and cortactin which is a structural protein that is known to participate in E-cadherin adhesion assemblies in epithelial cells wherein it helps in stabilizing the adherens junction.[33] There was no interaction seen between Syk and vinculin which is a structural protein component of both adherens junctions and focal adhesions and thus acts in both cell-cell and cell-matrix interactions.[34] The ability of cortactin to strengthen cell-cell contacts and the ability of Syk to interact with contactin explains partly how Syk may be able to foster cell adhesion and prevent metastasis. Integrins are important agents in cell-cell and cell-matrix interaction. β1-integrins have shown to form large tumors and show increased metastasis to liver and lung, [35] in the course of their research they discovered that just like in haemopoietic cells integrin crosslinking leads to activation of Syk. The activation of Syk causes it to catalyse various cellular proteins leading to suppression of cell motility.[36]

Chakraborty et al. went on to show the relation between hypoxia/reoxygenation (H/R) and its effect on Syk-Lck interaction. Hypoxia/reoxygenation (H/R) is a common feature seen in growing tumours.[37, 38] Lck( Lymphocyte specific protein tyrosine kinase) is a Src family nonreceptor protein-tyrosine kinase that is present in T cells, B cells, and breast cancer tissues. p72Syk has been shown to activate p56Lck through tyrosine phosphorylation.[39]
MelCAM (Melanoma Cell Adhesion Molecule) is an immunoglobulin that is known for its cell adhesion properties.\textsuperscript{[40]} MelCAM has been shown to be a tumour suppressor in breast carcinoma.\textsuperscript{[41]} uPA, MMP-9, are associated with tumour metastasis as cellular matrix destroyers\textsuperscript{[42]} and VEGF helps in angiogenesis which facilitates tumour progression.\textsuperscript{[43]} The research aimed at finding whether Hypoxia/reoxygenation (H/R) had any role in Syk-Lck interaction and what effect did this interaction have on tumour suppressor factor MelCAM and tumour promoting factor uPA, MMP-9 and VEGF. The results of the study showed that Syk act as a negative regulator of H/R induced tumour progression whereas Lck acts as a positive regulator and H/R plays a crucial role in dictating the interaction between Syk and Lck. These findings were further correlated to expression of uPA, MMP-9, VEGF and MelCAM, wherein it was found that H/R induced Lck which in turn inhibited MELCAM and helped in expression of uPA, MMP-9, and VEGF which led to tumour progression. In contrast Syk expression led to decrease in the level of Lck, increase in level of MelCAM and decrease in level uPA, MMP-9, and VEGF. The in-vitro studies were further supported by in-vivo studies done on mice where the same data was obtained.\textsuperscript{[44]} Figure 2 shows a schematic representation of possible mechanisms through which Syk hinders metastasis.

![Figure 2](image_url)

**Figure 2**: Various mechanisms through which Syk suppresses tumour metastasis. NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells, Lck: Lymphocyte specific protein tyrosine kinase MMP-9: Matrix Metalloproteinase 9, uPA: Urokinase-type plasminogen activator, VEGF: Vascular Endothelial Growth Factor Receptor, MelCAM: Melanoma Cell Adhesion Molecule.

**Prevention of apoptosis**

The cells that showed presence of Syk either naturally or via transfection had a common factor in them, that both the type of cells showed decreased metastatic nature but did not
show any change in their apoptosis behaviour. This raised a question that whether Syk was inhibiting metastasis while simultaneously inhibiting proteins and apoptotic factors that are responsible for cell death.

To study how Syk inhibits apoptosis of the tumour cells, Wang et al. conducted an experiment wherein they studied the effect of Syk on cell survival by evaluating its effect on the responses of cancer cells to induced stress by means of H$_2$O$_2$-induced apoptosis. It has previously been shown that Bcl-x$_L$ (B-cell lymphoma-extra large), a spliced transcript of BCL2L1 gene is necessary for cell survival.$^{[45]}$ Nucleolin is a protein that had been shown has a substrate for Syk.$^{[46]}$ They found that Syk acts as prosurvival factor by stabilizing the m-RNA for Bcl-x$_L$, since all cells that expressed Syk were found to show reduced inhibition of Bcl-x$_L$ after prolonged treatment with H$_2$O$_2$. The researchers have hypothesized that Syk activates nucleolin which in turn binds to AREs (Adenylate Uridylate Rich Elements) located in the 3’ UTR of Bcl-x$_L$, and also PABP(poly(A) binding protein) which ultimately leads to the stabilization of Bcl-x$_L$. They further stated that the binding of Syk to nucleolin probably occurs before activation of Syk, as it was seen that catalytically inactive Syk showed excellent binding.$^{[47]}$

Zhou and Geahlen showed another pathway through which Syk prevents apoptosis of cancerous breast epithelial cells. In their research they showed the interaction of Syk with TRIP (TNF Receptor-associated Interacting Protein). TRIP is a 53 kDa protein that binds to TRAF (TNF Receptor Associated Factor).$^{[48]}$ TRIP is responsible for antagonising TNF mediated activation of NF-kB. Takada and Aggarwal had previously shown the Syk enhances TNF-dependent activation of NF-kB$^{[49]}$ and NF-kB has been previously implicated in inhibition of TNF-mediated activation of NF-kB in HEK293 cells and HeLa cells.$^{[50]}$ Seeing that both Syk and TRIP modulated the same system Zhou and Geahlen set about to see the effect of these two modulators on NF-kB in cancerous breast cells of epithelial origin. Their study found out cells that express Syk tend to show greater activation of NF-kB as compared with cells that have low expression of Syk. They stated that Syk acts as a positive modulator for NF-kB whereas TRIP serves as negative modulator for NF-kB so the increase in the level of Syk tends to downregulate the expression of TRIP. They explained the mechanism of the interaction by proposing that Syk tends to phosphorylate TRIP which leads to its inactivation and prevents it from suppressing TNF-mediated activation of NF-kB.$^{[51]}$ Figure 3 shows ways by which Syk inhibits cell apoptosis.
Genetic changes modulating Syk expression

The fact many invasive cell lines showed the absence of Syk and also a progressive loss in Syk is seen as breast cancer moves from benign to invasive. This prompted researchers to ask if an alteration in the genetic pathway was going on in the cells which led to the downregulation of Syk.

Yuan et al. carried out a study to determine the reason for the loss of Syk in breast cancer cells. They studied the 5’CpG island methylation of SYK gene in breast cancer cell lines as it had been previously reported that methylation leads to inhibition of tumor suppressor genes.\textsuperscript{[52, 53]} In their study they correlated the SYK gene methylation status of various breast cancer cell lines with their relative Syk expression. Their studies found that the cell lines which were negative for Syk had high presence of 5’ CpG islands in their SYK gene as compared to cell lines which were positive for Syk. They found that treatment of cells with 5 -aza-2 -deoxycytidine a methylation inhibitor led to reactivation of Syk expression in Syk negative cells. They further demonstrated by using methylation specific PCR that out of 37 selected tumor cell lines 12 to be hypermethylated (32%). SYK gene methylation was also found out to be independent of prognostic markers such as estrogen receptors or HER2/neu, but a significant correlation between SYK gene expression and tumor grade was not seen. These findings go on to show that the loss of Syk expression is mediated at transcriptional level and methylation of SYK gene plays a very important part in this phenomenon.\textsuperscript{[54]}

Wang et al. reported the presence of a smaller spliced variant of Syk –Syk(S) which differs from the normal version of Syk- Syk (L) by lacking 23 amino acid del residue within the interdomain b. It was found that the Syk (L) could enter cell nucleus owning to the presence of an intact interdomain b region whereas Syk (S) was fully confined to cytoplasm of the cell. The mobilization of Syk into the nucleus is necessary for tumor suppression. This result showed that the expression of Syk was higher in mammary tumor cells as compared to cells
of normal mammary cells. It is strongly believed that in tumor cells an isoform switching occurs which tends to convert Syk (L) to Syk (S) which are incapable of preventing tumorogenesis and metastasis.[55]

Wang et al. in continuation of their past work,[55] tried to find out the role of Syk (L) in the nucleus. Given the fact that many proteins within the nucleus are responsible for gene transcription. The researchers tried to find that whether Syk performs similar function within the nucleus. Their work found that Syk (L) present in the nucleus was responsible for down regulation cyclin D1 and Fra1 oncogenes. Both these oncogenes have been previously implicated in progression of cancer.[56, 57, 58] The down regulation was hypothesized to occur by binding of Syk to Sp1 which is a transcription factor that is present in the promoter region of these oncogenes. The binding of Syk with sp1 causes repression of Sp1-activated transcription with the help of HDACs (Histone Deacetylase’s), which help in chromatin remodelling of Sp1 activated genes.[59]

CONCLUSION

From the above discussed material it is more than evident that Syk plays a very crucial role in suppression of metastasis in breast cancer via various mechanisms such as activation of NF-kB, upregulation of calpastatin, strengthening of cell contacts etc. The means through which Syk plays a role in modulation of NF-kB needs to be further evaluated as the reports currently available in literature are of a contradictory nature.

The role of Syk in other types of cancer and the effector molecules that are activated or deactivated by its action also needs to be extensively studied in order to facilitate a better understanding of Syk in cancer and in creation of pharmacological entities that would have a very selective role in upregualtion or downregulation of Syk, depending on the type of cancer to be treated.

REFERENCES


