Expression of MicroRNA-155 and Suppressor of Cytokines Signaling 1 (SOCS1) mRNA in Plasma Breast Cancer Patients

Dwi Nur Indah Sari[1], Dina Rahmina[2], Sumadi Lukman[3], Artanto Wahyono[3], Indwiani Astuti[4], Sofia Mubarika[5] and Teguh Aryandono[3]

[1]*Department of Biomedicine, School of Dentistry, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia*

[2]*Graduate Student Biomedical Science, Faculty of Medicine, Nursing and Public Health, Universitas Gadjah Mada, Yogyakarta, Indonesia*

[3]*Department of Surgery, Faculty of Medicine, Nursing and Public Health, Universitas Gadjah Mada, Yogyakarta, Indonesia*

[4]*Derpartment of Pharmacology and Therapy, Faculty of Medicine, Nursing and Public Health, Universitas Gadjah Mada, Yogyakarta, Indonesia*

[5]*Department of Histology and Cell Biology, Faculty of Medicine, Nursing and Public Health, Universitas Gadjah Mada, Yogyakarta, Indonesia*

Keywords: Breast cancer, plasma, microRNA-155, SOCS1 mRNA.

Abstract: Breast cancer is the most common cancer in women worldwide. miR-155 has been discovered to have an important role in the development of breast cancer. miR-155 targets many mRNAs of tumor suppressor genes, one of them is SOCS1. The discovery of miRNA in body fluids provides a novel alternative for the development of minimally invasive biomarker in cancer. However, limited study has been reported on the expression of miR-155 and SOCS1 mRNA in plasma breast cancer patients especially in Indonesian population.This study analyzed expressions of miR-155 and SOCS1 mRNA in plasma breast cancer patients This study was conducted using cross-sectional design, 32 plasma samples were collected from Dr.Sardjito Hospital. RNA was extracted from plasma and then cDNA was synthesized from RNA samples. Real-time qPCR was used to detect expression of miR-155 and mRNA SOCS1. Both expression was analyzed using Livak's method. The significance of the results were tested statistically using independence T-test. Expression of miR-155 at advanced stage was 2.43 higher than the early stages (p=0.047), while the mRNA expression of SOCS1 at advanced stage was 1.29 lower than early stage (p=0.170). Expression of miR-155 in ER, PR, & HER2 status revealed differences among subgroups, while the mRNA expression of SOCS1 was not significantly different. Other result showed that miR-155 expression was higher in triple negative tumor, while SOCS1 higher in luminal A tumor. Nevertheless, the difference was also not statistically significant (p value > 0,05). This study showed that expressions of miR-155 and SOCS1 mRNA was deregulated in breast cancer plasma and it believed to have an important role in breast cancer.

[1] https://orcid.org/0000- 0003-4691-9840
[2] https://orcid.org/0000- 0003-0125-8604
[3] https://orcid.org/0000- 0002-2607-6682
[4] https://orcid.org/0000- 0001-6870-4475
[5] https://orcid.org/0000- 0001-7008-9192
[6] https://orcid.org/0000- 0001-7205-652X
[7] https://orcid.org/ 0000-0003-4998-0006

Indah Sari, D., Rahmina, D., Lukman, S., Wahyono, A., Astuti, I., Mubarika, S. and Aryandono, T.
Expression of MicroRNA-155 and Suppressor of Cytokines Signaling 1 (SOCS1) mRNA in Plasma Breast Cancer Patients.
DOI: 10.5220/0010490402230230
In *Proceedings of the 1st Jenderal Soedirman International Medical Conference in conjunction with the 5th Annual Scientific Meeting (Temilnas) Consortium of Biomedical Science Indonesia (JIMC 2020)*, pages 223-230
ISBN: 978-989-758-499-2

1 INTRODUCTION

MicroRNAs (miRNA) are short non-coding RNA molecules that play important role as regulators of various cellular processes in post-transcriptional process (Ying et al. 2008). The role of miRNA is mediated by inhibition of translation or target mRNAs degradation. The mechanism of interaction between miRNA and mRNA is based on sequence complementation (Iorio & Croce 2009). Several biological processes are modulated by miRNAs, therefore deregulation of miRNAs expression is associated with various disease. Deregulation of miRNA expression has also been widely reported in various cancers, including breast cancer (Singh & Mo 2013; Iorio & Croce 2012; Ventura & Jacks 2009; Garzon et al. 2006).

Based on the target mRNAs, miRNA are divided into oncogenic miRNAs (oncomirs) and tumor suppressive miRNAs (Cho, 2011; Garzon et al. 2006). miR-155 plays as oncomir, and its overexpression is frequently reported in a number of malignant diseases (Higgs & Slack 2013; Johansson et al. 2013; Zhang et al. 2013; Jiang et al. 2012; Kong et al. 2010; Faraoni et al. 2009). Overexpression of miR-155 is reported during early development and progression of breast cancer. The expression of miR-155 has been studied that it has correlation with SOCS1 expression. It was reported that SOCS1, tumor suppressor gene, become target of miR-155 (Zhao et al. 2013; Huang et al. 2013; Cho, 2011; Jiang et al. 2010).

SOCS1 (Suppressor of cytokines signaling 1) is a negative feedback pathway regulator of Janus-activated kinase (JAK) / signal transducer and activator of transcription signaling (STAT) (Fujimoto & Naka 2010; Croker et al. 2009). Activation of JAK / STAT stimulates cells to proliferate, migrate or undergo apoptosis (Murray 2007; Rawlings et al. 2004). In the mammary gland, JAK / STAT plays role in the development of the mammary gland (particularly JAK1 and JAK2). Uncontrolled activation of JAK / STAT lead to tumorigenesis (Santillán-Benítez et al. 2014; Wagner & Schmidt 2011). SOCS1 regulates activation of JAK / STAT in order to maintain breast cells in homeostasis.

The initial study of the miR-155 and SOCS1 was reported in pancreatic cancer cells by Huang et al. (Huang et al., 2013). They reported that miR-155 played an important role in the regulation of invasion and migration of cancer cells via modulation of STAT3 and SOCS1 (Huang et al. 2013). The discovery of miRNA in body fluids represents a new alternative for the development of minimally invasive biomarker in breast cancer in addition to the use of Ca-153 which is not specific (Zheng et al. 2011; Corcoran et al. 2011; Brase et al. 2010). Previous studies showed that the expression of miR-155 in the serum of breast cancer patients was associated with clinical stages, molecular types, proliferation index and p53 expression (Zeng et al. 2014; Zheng et al. 2012). However, no study reported the expression of miR-155 and SOCS1 mRNA in the plasma of breast cancer patients especially for the Indonesian population. Expressions of miR-155 and SOCS1 mRNA from plasma of breast patients were performed in this study.

2 MATERIALS AND METHODS

This study was designed as observational study and cross sectional study.

2.1 Sample

Plasma samples of breast cancer patients were obtained from Dr. Sardjito Hospital. Samples were collected from patients that clinically and pathologically confirmed have breast cancer, not have another cancer, have not received any treatment, and aged 30-70 years.

Table 1: Characterics of samples

Characteristic	N	%
Stage		
Early stage (I-II)	10	31,25%
Advanced stage (III-IV)	22	68,75%
Receptor		
ER Positive	17	60,71%
ER Negative	11	39,29%
PR Positive	13	50,00%
PR Negative	13	50,00%
HER2 Positive	10	40,00%
HER2 Negative	15	60,00%
Molecular subtype		
Luminal A	10	35,71%
Luminal B	8	28,57%
HER2 overexpression	4	14,28%
Triple negative	6	21,42%

2.2 RNA Extraction and cDNA Synthesis

RNA extraction was performed using RNA Isolation Kit miRCURY-Biofluid according to the manufacturer' protocol. After RNA isolation, cDNAs

were synthesised using cDNA Synthesis kit II, 8-64 rxns. Each reaction was incubated 42°C for 60 minutes, 95°C for 5 minutes and followed by indefinite incubation at 4°C in PCR themal cycler (Biorad c1000).

2.3 Real Time-quantitative PCR (qPCR) of miR-155

qPCR was performed using ExiLent SYBR Green master mix (Cat No. 203402, Exiqon) and miRCURY LNATM Universal RT microRNA PCR for hsa-miR-155-5p. one reaction was mixed according to the manufacturer's protocol. Each reaction was then incubated with following programs : denaturation 95°C for 10 minutes, 40 cycles amplification : 95ºC, 58ºC for 10 seconds in Biorad CFX 96. MiR-16 was used as reference gene for miR-155-5p quantification. Relative expression was calculated according to the comparative Livak's Method : $2^{-\Delta\Delta Cq}$. If the fold change <1, value of fold decreased was calculate by 1/fold change.

2.4 Real Time PCR mRNA SOCS1

One-Step qRT-PCR using KAPA™ SYBR® kit was used to analyze SOCS1 mRNA expression. One reaction was mixed according to the manufacturer's protocol. qPCR program were performed on Biorad CFX 96. cDNA synthesis 45ºC for 5 minutes, RT inactivation 95°C for 3 minutes, denaturation 95° C for 10 s, annealing for 30 seconds 59,4°C amplification of 45 cycles : 95°C for 10 seconds and 59 , 4 °C for 30-second ramp-rate 1,6°C / s Optical read and melting curve analysis. Beta Actin mRNA was used as reference gene for SOCS1 mRNA quantification. Relative expression was calculated according to the comparative Livak's Method : $2^{-\Delta\Delta Cq}$. If the fold change <1, value of fold decreased was calculate by 1/fold change.

2.5 Statistical Analysis

All of the miR-155 and SOCS1 expression differences among breast cancer subgroup was then analyzed statistically using Independent T-tests. Statistical significance was determined when P value ≤ 0.05.

3 RESULT

3.1 Interaction Analysis of miR-155 with mRNA SOCS1 (in silico)

Interaction analysis of miR-155 with its target mRNA was carried out through bioinformatic analysis on miRanda/mirtarbase (www.mirtarbase.mbc.nctu.edu.tw). One of the targets for mir-155 is mRNA SOCS1. The SOCS1 mRNA sequence has a length of 1216 base pairs with the region predicted to interact with miR-155 being in the 3'UTR (untranslated region) of SOCS1 mRNA. Possible interaction of miR-155 with 3'UTR mRNA SOCS1 occur at three site, at 15-34 nucleotides withal Minimum Free Energy (MFE) -15,50 kj/mol, at 218-242 nucleotides withal MFE -15,90 kj/mol and at nucleotides 404-425 withal MFE -9,13 kj/mol (see figure 1).

TGACCGGCAGCGCCC**GCCGTGCACGCAGCATTAAC**
TGGGATGCCGTGTTATTTTGTTATTACTTGCCTGG
AACCATGTGGGTACCCTCCCCGGCCTGGGTTGGAG
GGAGCGGATGGGTGTAGGGGCGAGGCGCCTCCCGC
CCTCGGCTGGAGACGAGGCCGCAGACCCCTTCTCA
CCTCTTGAGGGGGTCCTCCCCCTCCTGGTGCTCCC
TCTGGGT**CCCCCTGGTTGTTGTAGCAGCTTAA**CTG
TATCTGGAGCCAGGACCTGAACTCGCACCTCCTAC
CTCTTCATGTTTACATATACCCAGTATCTTTGCAC
AAACCAGGGGTTGGGGGAGGGTCTCTGGCTTTATT
TTTCTGCTGTGCAGAATCCTATTTTATATTTTTTA
AAGTCAGTTTAGGTAATA**AACTTTATTATGAAAGT**

Figure1. Binding site of miR-155 on 3'UTR region mRNA SOCS1

3.2 Expression of miR-155 and mRNA SOCS1

Expressions of miR-155 and SOCS1 mRNA were analyzed by qRT-PCR as previously described. Expression of miR-155 was calculated defined from Cq value. Cq value represents first cycle in PCR when the quantitative graph increased exponentially. The small Cq value means concentration of PCR product is high in the sample. Then Cq value were used to calculated the expression of miR-155 and SOCS1 mRNA using Livak's Method. The fold change (FC) value could upregulated (FC>1) or downregulated (FC<1). If the fold change <1, value of fold decreased was calculate by 1/fold change.

Expression of miR-155 in advanced stage of breast cancer patients was significantly higher than

early stage. Expression of miR-155 in advanced stage of breast cancer was 2.43 times higher than the early stage. In addition, expression of SOCS1 in early stage was higher than advanced stage. Fold change expression of SOCS1 in advanced stage of breast cancer was 0.77 times to early stage. It was mean expression of SOCS1 in advanced stage of breast cancer was 1.29 fold decreased from early stage However, the differences were not statistically significant (See Table 2).

Table 2: miR-155 and SOCS1 mRNA expression according to clinical stage

Sample	Mean (ΔCq)	ΔΔ*Cq*	*Fold change* ($2^{-\Delta\Delta Cq}$)	*P value*[#]
miR-155				
Advanced stage	11.51	-1.28	2.43 (*up-regulated*)	0.047
Early stage	12.79			
SOCS1 mRNA Advanced stage	4.90	0.38	0.77 (*down-regulated*)	0.170
Early stage	4.53			

[#] p value were analyzed with independent *t-test*

We subclassified samples by their estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor. Expression of miR-155 was not significantly different between ER + and ER -. Fold change expression miR-155 in PR - was 0.76 times to PR +. It was mean that expression miR-155 in PR - was 1.31 fold decreased from PR +. Expression of miR-155 in HER2 - was higher 2.55 times from HER2 +. However, the different miR-155 expression was not statistically significant (see Table 3).

The expression of SOCS1 mRNA was also analyzed according ER, PR, and HER2 status. The results showed that expression of SOCS1 were not different in both in ER + and ER - and also in HER2 + and HER2 -. But in PR - was slightly lower than in PR +. However, that were not statistically different between different ER, PR, and HER2 status (see Table 4).

Subtypes of breast cancer have been implicated to determine prognosis. We subclassified breast cancer patients according to the subtypes. Our result showed expression of miR-155 was higher in triple negative than the other subtypes, while the highest expression of SOCS1 mRNA was in Luminal A subtype and the lowest was in triple negative subtype.

Then, we compared expression of miR-155 and SOCS1 mRNA between Luminal A and other subtypes. The result showed that miR-155 in Luminal A was upregulated/higher than Luminal B dan HER2 overexpressions subtype, but lower than Triple negative subtype. However, the differences were not statistically significant. Our result also showed that expression of SOCS1 mRNA in Luminal A subtype was higher than in the other subtypes but that were not statically significant.

Table 3. miR-155 expression according to ER, PR, HER2

Sample	Mean (ΔCq)	ΔΔ*Cq*	*Fold change* ($2^{-\Delta\Delta Cq}$)	*P value*[#]
ER-	11.47	-0.17	1.13	0.838
ER+	11.64			
PR-	11.84	0.39	0.76 (*downregulated*)	0.648
PR+	11.45			
HER2-	11.17	-1.35	2.55 (up*regulated*)	0.074
HER2+	12.52			

[#] p value were analyzed with independent *t-test*

Table 4. SOCS1 mRNA expression according to ER, PR, and HER2 expression

Sample	Mean (ΔCq)	ΔΔ*Cq*	*Fold change* ($2^{-\Delta\Delta Cq}$)	*P value*
ER-	4.74	0.01	0.99	0.994
ER+	4.73			
PR-	5.04	0.32	0.80 (*down-regulated*)	0.482
PR+	4.72			
HER2-	4.87	-0.10	1.07	0.830
HER2+	4.97			

[#]p value were analyzed with *independent t-test*

Table 5 miR-155 expression in subtype

Subtype	Mean (ΔCq)	ΔΔ*Cq*	*Fold change* ($2^{-\Delta\Delta Cq}$)	P value[#]
Luminal A	10.94	-0.68	1.60 (*up-regulated*)	0,506
Luminal B	11.62			
Luminal A	10.94	-0.94	1.92 (*up-regulated*)	0,547
HER2 overexpression	11.88			
Luminal A	10.94	0.92	0.53 (*down-regulated*)	0,461
Triple negative	10.02			

[#]p value were analyzed with *independent t-test*

Table 6 SOCS1 mRNA expression in subtype

Subtype	Mean (ΔCq)	ΔΔ*Cq*	*Fold change* ($2^{-\Delta\Delta Cq}$)	*P value*[#]
Luminal A	4.59	-0.27	1.21 (*up-regulated*)	0,570
Luminal B	4.86			
Luminal A	4.59	-0.57	1.48 (*up-regulated*)	0.353
HER2 over expression	5.16			
Luminal A	4.59	-0.61	1.52 (*up-regulated*)	0.380
Triple negative	5.20			

[#]p value were analyzed with *independent t-test*

4 DISCUSSION

MicroRNAs (miRNA) are short non-coding RNAs that play an important role in the regulation of gene expression post-transcriptionally (Ying et al. 2008). In bioinformatics analysis, miR-155 targets many genes, one of which is SOCS1 mRNA. miR-155 interacted with SOCS1 mRNA at 3'UTR. The strongest interaction between miR-155 and SOCS1 mRNA might be in 218-242 base of 3'UTR SOCS1 which was indicated by the most negative mfe value. Ragan et al. (2011) reported that the mfe value indicates the free energy used to break the structure of the interactions that occur between miR and target mRNA. The more negative mfe value will make it difficult to break this structure. This means that the structure has high stability (Chan & Zhang, 2009). The high stability of the miR-mRNA interaction structure allows the role of miR-155 in the regulation of SOCS1 mRNA expression.

Our study has been performed with plasma of breast cancer patients and the expression of microRNA can be detected in this samples. It showed that microRNA in plasma was relatively stable and provides to be potential alternative for minimal invasive cancer biomarkers (Zheng et al. 2011; Kosaka et al. 2010). MiRNAs are relatively stable in body fluids because miRNAs are protected in lipid complex or lipoprotein such as apoptotic bodies, microvesicle, or exosome (Cortez et al. 2011; Kosaka et al. 2010) or presence in a modified structure (methylation, adenylation, uridilasi) (Chen et al. 2012).

Our results also showed SOCS1 mRNA can be detected in plasma samples. This might occur because SOCS1 mRNA was also protected within microvesicle (Kosaka et al. 2010; Marini et al. 2006).

In this study, expression of miR-155 was significantly higher (2.43 times) at an advanced stage than early stages of breast cancer patients (p=0,047). Other study showed overexpression of miR-155 both at an early stage and advanced stage breast cancer compared with healthy controls. In the advanced stage breast cancer, expression of miR-155 was significantly higher compared with than early stages ($p < 0.05$) (Wang et al. 2012). Studies by Zheng et al. and Liu et al showed increased expression of miR-155 in breast cancer patients. They found that the expression of miR-155 correlated with proliferation index, lymph node infiltration, and advanced stage (Zheng et al. 2012; Zeng et al. 2014).

miR-155 has been reported as regulator in the growth stimulating factor and metastasis (Sun et al. 2012). The role of microRNA in metastasis is suggested by the presence of microRNA within exosome in circulation. Exosomal microRNAs plays a role in the intercellular communication (Schwarzenbach et al. 2014; Cortez et al. 2011). Plasma miR-155 is thought to be uptaked by recipient cells, causing changes in the expression of mRNA targets of miR-155 in these cells (Cheng, 2015). The cancer cells might be utilized miRNA to influence their development and has been reported to have a strong correlation with cancer invasion and metastasis (Sun et al., 2012; Cheng et al., 2015).

One of the miR-155 targets is SOCS1 mRNA. SOCS1 is a major regulator JAK-STAT pathway that affects expression of various genes involved in cancer progression (proliferation, invasion, migration, apoptosis resistance, and angiogenesis) (Huang et al. 2013). Our study showed that expression of SOCS1 at an advanced stage was lower than in the early stage of breast cancer. Our results were similar with a study by Sasi et al, that SOCS1 expression decreased in the higher-stage breast cancer and positively correlated with the clinical outcome of patients (Sasi et al. 2010). Jiang et al. (2010) reported that in breast cancer cell line, the decreased expression of SOCS1 was thought to be due to SOCS1 mRNA being targeted by miR-155. Huang et al. (2013) reported that miR-155 plays an important role in regulating the invasion and migration of cancer cells by decreasing SOCS1 expression, resulting in continuous STAT3 activation.

In this study, we also demonstrated that expression of miR-155 in different of ER, PR and HER2 status. The result was not statistically different both ER status. miR-155 was higher in PR + than PR- breast cancer, and was higher in HER2- than HER2 + breast cancer. Expression of miR-155 on PR + might be affect the growth of breast cancer. Tanos et al.

(2012) reported that through binding to its receptor, progesterone induces STAT3 activation in breast cancer via the JAK and Src pathways. Absence or reduction of negative regulator (SOCS1), resulting in continuously active STAT3 leading to cancer progression. However, this result is not supported by fold change of SOCS1 mRNA. This might occur because miR-155 targets other PR-related mRNAs.

Expression of SOCS1 in both PR+ and PR- breast cancer was similiar in our study. This result was different with the study conducted by Sun et al. Sun and colleagus showed that progesterone up regulated SOCS1 expression through induction of LPS (Sun et al. 2012).

In this study, miR-155 expression appeared to be higher in patients with HER2- than HER2+, whereas SOCS1 mRNA expression was almost the same in both HER2 states. Bischoff et al. (2015) reported that miR-155 might be act as a negative regulator of HER2 signaling. miR-155 was reported to decrease HER2-induced Akt activity, but miR-155 played a role in increasing basal Akt activity. This is in line with reports that miR-155 is positively correlated with basal PI3K activation by targeting PI3K negative regulators (p85α and SHIP) (Huang et al., 2013), and upstream negative regulator PI3K (SOCS1) (Jiang et al., 2010).

In subtype breast cancer, our result showed that expression of miR-155 was elevated in Triple-negative breast cancer. However, the result showed not statistically significant. This result was same as result of a study by Blenkiron et al. (2007) that documented elevated miR-155 expression in the triple negative breast cancer. Lu et al (2012) also reported that expression of miR-155 in plasma also showed significant differences among the subtypes of breast cancer with the highest expression was seen in triple negative subtype followed by HER2 overexpression, Luminal B, and Luminal A ($p = 0.027$).

In Triple negative breast cancer, expression of ER, PR, and HER2 is negative. A microRNA profiling study by Gasparini et al. (2014) showed that there were 4 miRNAs that were correlated with triple negative subtypes, one of them was miR-155. The high expression of miR-155 in triple negative subtype might be associated with BRCA1 (Chang & Sharan 2012). BRCA1 is a gene that is frequently mutated in breast cancer, especially in the triple negative subtype (Bange et al. 2001). Davis et al reported that silencing of BRCA1 leads to reduced expression of ER and PR (Davis et al , 2014).

SOCS1 expression was higher in luminal A. The luminal subtype is the subtype with the best prognosis. This is in accordance with Sasi et al. (2010) who reported that the expression of SOCS1 was positively correlated with good outcomes (the lowest recurrence and the lowest mortality rate). (Sasi et al. 2010).

5 CONCLUSIONS

In plasma of breast cancer patients, expression of miR-155 in advanced stage was higher than in the early stages, while the expression of SOCS1 mRNA in advanced stage was lower than the early stage. MiR-155 was differentially expressed according to ER, PR, HER2 while the expression of SOCS1 was not different according to ER, PR, HER2. miR-155 expression was higher in triple negative, while SOCS1 was higher in luminal A. However, the differences were not statistically significant. Deregulation of miR-155 dan SOCS1 expressions is believed to have important role in breast cancer progressivity.

ACKNOWLEDGEMENTS

This study was support by a grant from the PUPT 2014 grants, Universitas Gadjah Mada. Researchers supervisor: Prof. dr. Sofia Mubarika H., M.Med.Sc, Ph.D and Prof. Dr. dr. Teguh Aryandono Sp.B (K) Onk; Research Advisor: dr. Artanto Wahyono, Sp.B., Dr.med. dr. Indwiani Astuti; Prof. Dr. Mustafa, Apt., Kes; dr. Ahmad Hamim Sadewa, Ph.D., Drs. Zulaela, Dipl. Med. Stat., M.Si. dr. Ahmad Ghozali, Sp.PA(K) for analized immunohistochemistry data's patients. Laboratory supervisor : Risky Oktriani, S. Si, M. Biotech, dr. Sumadi Lukman A., Ph.D., dr. Zulrachman Erlangga, Nihayatus Saadah, S. Si, M.Sc. and the entire members of the study group mikroRNA (genomiR).

.REFERENCES

Bange, J., Zwick, E. & Ullrich, A., 2001. Molecular targets for breast cancer therapy and prevention. *Nat Med*, 7(5), 548–52.

Berishaj, M., Gao, S. P., Ahmed, S., et al., 2007. Stat3 is tyrosine-phosphorylated through the interleukin-6 / glycoprotein 130 / Janus kinase pathway in breast cancer. *Breast Cancer Res*, 9(3), 1–8.

Bischoff, A., Bayerlova, M., Strotbek, M., Schmid, S., Beissbarth, T., & Olayioye, M. O. 2015. A global microRNA screen identities regulators of the ErbB

receptor signaling network. *Cell Comm Sig*. 13 (5): 1-15.

Blenkiron, C., Goldstein, L. D., Thorne, N. P., et al., 2007. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. *Genome Biol*, 8(10), 214.1–.16.

Brase, J.C., Wuttig, D., Kuner, R., et al., 2010. Serum microRNAs as non-invasive biomarkers for cancer. *Mol Cancer*, 9(1), 1–9.

Chan, H. S., & Zhang, Z. 2009. Liaison amid disorder: non-native interactions may underpin long-range coupling in proteins. *Journal of Biology*. 8(3): 27.

Chang, S. & Sharan, S.K., 2012. BRCA1 and microRNAs: emerging networks and potential therapeutic targets. *Mol. Cells*, 34(5), 425–32.

Chen, X., Liang, H., Zhang, J., et al., 2012. Secreted microRNAs: a new form of intercellular communication. *Trends in cell biology*, 22(3), 125–32.

Cheng, G. 2015. Circulating miRNAs: roles in cancer diagnosis, prognosis and therapy. *Advanced Drug Delivery Reviews*. 81: 75–93.

Corcoran, C., Friel, A. M., Duffy, M. J., et al., 2011. Intracellular and extracellular microRNAs in breast cancer. *Clin Chem*, 57(1), 18–32.

Cortez, M.A., Bueso-ramos, C., Ferdin, J., et al., 2011. MicroRNAs in body fluids—the mix of hormones and biomarkers. *Nat Rev Clin Oncol*, 8(8), 467–77.

Croker, B.A., Kiu, H. & Nicholson, S.E., 2009. SOCS regulation of the JAK/STAT signaling pathway. *Semin Cell Dev Biol*, 19(4), 414–22.

Faraoni, I. Antonetti, F. R., Cardone, J., et al., 2009. MiR-155 gene: a typical multifunctional microRNA. *Biochim Biophys Acta*, 1792(6), 497–505.

Fujimoto, M. & Naka, T., 2010. SOCS1 , a negative regulator of cytokine signals and TLR responses , in human liver diseases. *Gastroenterol Res Pract*, 2010, 1–7.

Garzon, R. Marcucci, G., & Croce, C. M. 2006. MicroRNA expression and function in cancer. *J Mol Med*, 12(12), 580–7.

Gasparini, P., Cascione, L., Fassan, M., & Lovat, F. 2014. MicroRNA expression profiling identifies a four microRNA signature as a novel diagnostic and prognostic biomarker in triple negative breast cancers. *Oncotarget*. 5(5): 1174–84.

Gupta, N. & Mayer, D., 2013. Interaction of JAK with steroid receptor function. *JAK-STAT*, 2, 37–41.

Higgs, G. & Slack, F., 2013. The multiple roles of microRNA-155 in oncogenesis. *J Clin Bioinformatics*, 3, 1–8.

Huang, C. Li, H., Wu, W., et al., 2013. Regulation of miR-155 affects pancreatic cancer cell invasiveness and migration by modulating the STAT3 signaling pathway through SOCS1. *Oncol Rep*, 30, 1223–30.

Iorio, M. V & Croce, C.M., 2012. MicroRNA dysregulation in cancer : diagnostics , monitoring and therapeutics . A comprehensive review. *EMBO Mol Med*, 4, 143–59.

Iorio, M. V & Croce, C.M., 2009. MicroRNAs in cancer: small molecules with a huge impact. *J Clin Oncol*, 27(34), 5848–56.

Jiang, S. et al., 2012. A novel miR-155/miR-143 cascade controls glycolysis by regulating hexokinase 2 in breast cancer cells. *EMBO J*, 31(8), 1985–98.

Jiang, S., Zhang, H. & Lu, M., 2010. MicroRNA-155 functions as an oncomiR in breast cancer by targeting the Suppressor of Cytokine Signaling 1 gene. *Cancer Res*, 70, 3119–27.

Johansson, J. et al., 2013. MiR-155-mediated loss of C/EBPβ shifts the TGF-β response from growth inhibition to epithelial-mesenchymal transition, invasion and metastasis in breast cancer. *Oncogene*, 32(50), 5614–24.

Kong, W. He, L., Coppola, M., et al., 2010. MicroRNA-155 regulates cell survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. *The Journal of biological chemistry*, 285(23), 17869–79.

Kosaka, N., Iguchi, H. & Ochiya, T., 2010. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci*, 101(10), 2087–92.

Liu, J. Mao, Q., Liu, Y., et al., 2013. Analysis of miR-205 and miR-155 expression in the blood of breast cancer patients. *Chin J Cancer Res*, 25(1), 46–54.

Lu, Z. Ye, Y., Jiao, D., et al., 2012. MiR-155 and miR-31 are differentially expressed in breast cancer patients and are correlated with the estrogen receptor and progesterone receptor status. *Oncology Letters*, 4(5), 1027–32.

Marini, A. et al., 2006. Epigenetic Inactivation of Tumor Suppressor Genes in Serum of Patients with Cutaneous Melanoma. *J Invest Dermathol*, 126, 422–431.

Martin, E.C. Rhodes, L. Elliot, S., et al., 2014. microRNA regulation of mammalian target of rapamycin expression and activity controls estrogen receptor function and RAD001 sensitivity. *Mol Cancer*, 13(1), 1–13.

Murray, P.J., 2007. The JAK-STAT signaling pathway: input and output integration. *J Immunol*, 178,.2623–29.

Ragan, C., Zuker, M., Ragan, M.A. 2011. Quantitative prediction of miRNA-mRNA interaction based on equilibrium concentrations. *PLoS Compt Biol*. 7 (2): e10001090.

Rawlings, J.S., Kristin, M. & Harrison, D.A., 2004. The JAK / STAT signaling pathway. *J Cell Sci*, 1(117), 1281–83.

Santillán-Benítez, J.G. Mendieta-Zeró, H., Gómez-Oliván, L. M., et al., 2014. JAK2, STAT3 and SOCS3 gene expression in women with and without breast cancer. *Gene*, 547(1), 70–6.

Sasi, W. Jiang, W. G., Sharma, A., et al., 2010. Higher expression levels of SOCS 1 , 3 , 4 , 7 are associated with earlier tumour stage and better clinical outcome in human breast cancer. *BMC Cancer*, 10(178), 1–13.

Sasi, W. Sharma, A. K., Mokbel, K., et al., 2014. The role of Suppressors of Cytokine Signalling in human neoplasms. *Mol Biol Int*, 2014, 1–24.

Schwarzenbach, H., Nishida, N., Calin, G. A., et al., 2014. Clinical relevance of circulating cell-free microRNAs in cancer. *Nat Rev Clin Oncol*, 1–12.

Singh, R. & Mo, Y.-Y., 2013. Role of microRNAs in breast cancer. *Cancer Biol Ther*, 14(3), 201–12.

Sun, Y. Wang, M., Lin, G., et al., 2012. Serum microRNA-155 as a potential biomarker to track disease in breast cancer. *PloS One*, 7(10), 1–8.

Tanos, T., Rojo, L. J., Echeverria, P., & Brisken, C. 2012. ER and PR signaling nodes during mammary gland development. *Breast Can Res*. 14:210

Ventura, A. & Jacks, T., 2009. MicroRNAs and cancer: short RNAs go a long way. *Cell*, 136(4), 586–91.

Wagner, K.-U. & Schmidt, J.W., 2011. The two faces of Janus kinases and their respective STATs in mammary gland development and cancer. (published online)

Wang, H. Tan, G., Dong, L., et al., 2012. Circulating miR-125b as a marker predicting chemoresistance in breast cancer. *PloS one*, 7(4), 1–8.

Ying, S.-Y., Chang, D.C. & Lin, S.-L., 2008. The microRNA (miRNA): overview of the RNA genes that modulate gene function. *Mol Biotechnol*, 38(3), 257–68.

Zeng, H. Fang, C., Nam, S., et al., 2014. The clinicopathological significance of microRNA-155 in breast cancer: a meta-analysis. *BioMed research international*, 2014, 724209.

Zhang, C.-M., Zhao, J. & Deng, H.-Y., 2013. MiR-155 promotes proliferation of human breast cancer MCF-7 cells through targeting tumor protein 53-induced nuclear protein 1. *J Biomed Sci*, 20, 1–10.

Zhao, X. Zhang, W., Liang, H., et al., 2013. Overexpression of miR -155 Promotes Proliferation and Invasion of Human Laryngeal Squamous Cell Carcinoma via Targeting SOCS1 and STAT3. *PloS one*, 8(2), 1–11.

Zheng, D. et al., 2011. Plasma microRNAs as novel biomarkers for early detection of lung cancer. Int J *Clin Exp Pathol*, 4(6), 575–86.

Zheng, S.-R. Guo, G.-L., Zhang, W., et al., 2012. Clinical significance of miR-155 expression in breast cancer and effects of miR-155 ASO on cell viability and apoptosis. *Oncology reports*, 27(4), 1149–55..