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Tong-Cun Zhang
Pingkai Ouyang
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Bill Skarnes *Editors*

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Preface

2012 International Conference on Applied Biotechnology (ICAB2012) was organized by Tianjin University of Science and Technology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences was held from October 18th to 19th, 2012 in Tianjin, China.

The conference served as a forum for exchange and dissemination of ideas and the latest findings among all parties involved in any aspects of applied biotechnology. The following distinguished professors gave keynote speeches: Hassan Ashktorab (Howard University, U.S.A), William Carl Skarnes (The Wellcome Trust Sanger Institute, U.K), Hiroyuki Takenaka (Kyushu Kyoritsu University, Japan) and Xueli Zhang (Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, China). The conference was complemented by talks given by other 51 professors and investigates.

More than 200 authors from 44 different universities, institutes and companies submitted conference papers. A lot of fields have been covered, ranging from fermentation engineering, cell engineering, genetic engineering, enzyme engineering and protein engineering.

Special thanks are given to Academic Committee, Organizing Committee and Secretary Staff of the conference for the commitment to the conference organization. We would like also to thank all the authors who contributed with their papers to the success of the conference.

This Book gathers a selection of the papers presented at the conference; it contains contributions from both academic and industrial researchers providing a unique perspective on the research and development of applied biotechnology from all over the world. The scientific value of the papers also helps the researchers in this field to get more valuable results.

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Contents

Part III
Pharmaceutical Biotechnology

Chapter 66
Promising Biomarkers: MicroRNAs at Diagnosis, Therapy and Prognostic Evaluation of Breast Cancer

Dalin Lu, Nan Wang, Xinghua Liao, Xuan Huang, Jianhua Zhang, Zhenyu Wang, Lian Duan, Jiajie Liu, Baoshu Jin, Yue Wang and Tong-Cun Zhang

Abstract MicroRNAs (miRNAs) are small noncoding RNAs with regulatory functions, which play an important role in malignancies. An increasing amount of experimental evidence has shown that many miRNAs are aberrantly expressed in breast cancer and influence breast cancer behavior and progression. Furthermore, miRNAs can act either as tumor suppressors or as oncogenes, depending on the targets they regulate, and measurements of miRNAs expression in breast cancer have diagnostic and prognostic implications. Thus, this implies that miRNAs have huge potential as biomarkers. In addition, their extreme stability and ease of detection further support the idea that miRNAs have great potential to evolve into effective biomarkers in the clinic. The objective of this review is to update current realization regarding that miRNAs are promising candidates at diagnostic, therapeutic and prognostic evaluation aspects of clinical application.

Keywords Breast cancer · miRNAs · Diagnosis · Therapy · Prognosis

D. Lu, X. Liao, and X. Huang contributed equally to this work and are nominated as the first author.

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66.1 A Brief Introduction to MicroRNAs

'Noncoding' or 'non-messenger' RNAs are various molecules with structural, enzymatic, and regulatory functions. Among these RNAs with regulatory activity are miRNAs, which are about 22 nucleotides in length [1]. The majority of animal miRNAs are imprecisely matching to their target mRNAs, and then they hinder protein synthesis by unknown mechanisms. Some studies even suggest that the translationally repressed target mRNAs remain related with ribosomes [2–4]. In addition, computational algorithms have estimated that miRNAs could target more than 30 % of the human genome [5]. Significantly, a single miRNAs can regulate more than one gene, and equally a particular gene can be regulated by compound miRNAs. Thus, miRNAs, can regulate diverse biological processes, for example, development, angiogenesis, differentiation, immune cell function, proliferation, apoptosis and wound healing, and so on [6].

An increasing number of deregulated miRNAs has been detected in breast cancer, and many of them are closely associated with cancer metastasis and poor prognosis, and reduce survival time of breast cancer patients. For example, the high-expressed miRNA, miR-21, has been reported to be associated with advanced stage, node positivity. Meanwhile, miRNA-21 knock-down in cell-line models have been related to increased sensitivity to topotecan and taxol. All of them suggest an important role of miRNAs in oncogenesis, diagnosis, therapy, and prognosis of breast cancer [7–11].

In this review, we discuss miRNAs as potential biomarkers used in breast cancer, emphasizing that miRNAs can be effectively used in these aspects of diagnosis, therapy, and prognostic valuation in the clinical setting. Our efforts are to research and recommend more effective methods to improve the quality of life and survival time.

66.2 Potential Use of miRNAs as Biomarkers for Breast Cancer

miRNAs expression profiles derived from large-scale analyses of tumor samples have been shown to serve as phenotypic signatures of particular cancer types. For example, seven independent studies have analyzed the miRNA profile of breast cancer tissues or cell lines, compared to normal tissues or cell-lines. These studies described a general down-regulation of miRNAs in breast cancer tissues or cell lines compared to normal breast cancer tissues or cell-lines [7, 12–17].

Up to now, most profiling studies have paid attention to deregulated miRNAs either in the primary breast cancer tissue or in breast cancer cell lines. miRNAs expression profile analyses from all kind of breast cancer or cell lines analyzed by

miRNAs profiling have revealed considerably different miRNA profiles (for mature or precursor miRNAs) compared with normal cells from the same tissue. A review of the published large-scale miRNA profiles reveals that several constantly deregulated miRNAs in tumors from breast cancer patients together with their clinical correlations are listed in Table 66.1. Undeniably, many of these miRNAs, such as let-7a, miR-10b, miR-21, miR-31, miR-145, and miR-155 are deregulated in various types of cancer, including breast, prostate, colon, lung, liver cancers, and melanoma [17–21]. In addition, the overexpression of several miRNAs such as miR-21 and so on in human breast cancer is associated with advanced clinical stage, lymph node metastasis, and patient poor prognosis [14]. Furthermore, Zhao et al. have demonstrated that circulating miRNAs in plasma could potentially serve as novel minimally invasive biomarkers for early detection of breast cancer [22]. Heneghan and his colleagues surveyed a panel of 7 candidate miRNAs in whole blood RNAs from 148 breast cancer patients and 44 specific miRNA, miR-195, only high-expressed in breast cancer. Additionally, they observed a significant reduction of miR-195 in postoperative whole blood compared with the preoperative samples of the same patients [17]. Thus, it could be applied not only to directly detect breast tumors, but also perceive breast cancer relapse.

Besides, due to their resistance to degradation, blood miRNAs appear to be very stable, and there is budding interest in profiling circulating miRNAs either as diagnostic noninvasive agent markers or as therapeutic measures. Circulating miRNAs have been widely reported to be drastically elevated in the blood of cancer patients compared with healthy controls, and levels of these miRNAs are associated with the primary tumors. The elimination of the primary tumor leads to the recover of deregulated circulating miRNAs, implying that many of these elevated circulating miRNAs are 'tumor-derived' and cancer-specific [6]. The current belief is that these 'tumor-derived' circulating miRNAs are released from the primary tumor via exosome vesicles and apoptotic bodies although the exact mechanisms of release are still emerging [23–25]. Although there will be technical difficulties, all these miRNAs' properties imply that miRNAs as clinical biomarkers will have a promising prospect.

66.3 miRNAs Profiling to Advance Development of Diagnosis, Therapy, and Prognostic Evaluation

There is increasing evidence to support that miRNAs are closely associated with a huge proportion of breast cancer heterogeneity. Many miRNAs have been shown to be deregulated in breast cancer [7, 13, 26, 27] and specific miRNAs functioning as regulators of tumorigenicity, invasion, and metastasis have been documented [8, 9, 28, 29]. In addition, miRNAs that can regulate ER, PR, and HER2/neu,

Table 1 Commonly regulated miRNAs, their targets, and pathway in breast cancer

miRNA	Tumor expression level	References	Validated targets	Pathways	References
miR-21	↑	[7]	HER2, BCL2, TPM1, TIMP3, PDCD4, PTEN, MASPIN, RHOB, MMP3	Apoptosis, invasion, metastasis	[14, 28, 34–38]
miR-155	↑	[7]	Caspase3, FOXO3A, SOCS1, RHOA	Proliferation, TGF-β, signaling	[39–42]
miR-191	↑	[7, 43]			
miR-196a	↑	[7, 43]	ANXA1	Proliferation, apoptosis	[44]
miR-10b	↓	[7]	RHOC, TIAM, HOXD10	Migration, invasion, metastasis	[8, 45, 46]
miR-302b	↑	[47]	p21	Invasion, Migration	[47]
miR-425	↑	[9]	DICER1		[47]
miR-100	↓	[48]			
miR-125b	↓	[7, 43, 48]	HER2, HER3, CRAF, MUC1, BAK, ERA, RTKN	Proliferation, apoptosis, migration	[26, 30, 49, 50]
miR-145	↓	[7, 48]	TP53, PUMA, c-Myc,MUC1, ERα, RTKN	Proliferation, apoptosis, invasion	[51–54]
miR-205	↓	[26]	HER3, VEGF-A, EMT	Proliferation, invasion	[7, 55, 56]

This table represents miRNAs whose expressions are notably regulated in clinical samples (tumors of human patients)

known to be of diagnostic value, therapeutic worth, and prognostic significance in breast cancer, has been demonstrated [30, 31].

At present, miRNAs expression profiling can be used to evaluate clinicopathological variables to categorize breast tumors. To predict disease progression and prognosis using miRNAs expression profiling analysis is of particular interest. First of all, profiling features the potential to identify novel prognostic indicators, which may contribute to improve patients for adjuvant therapy. This approach has already shown promise with genomic signatures, and miRNA profiles appear to have superior accuracy to mRNA profiling [32]. Besides, the measurement of miRNAs with regulatory roles, distinct breast tumor samples could identify novel targets for therapeutic manipulation. For example, miRNA-221 and 222 are negatively correlated with ERα protein expression and the knockdown of miRNA-221/222 can effectively restore ERα protein expression [33]. Of course, there are more miRNAs associated with PR and HER2. Here, we do not list all of them.

In conclusion, all these findings discussed in this report suggest that miRNAs detected are promising as novel biomarkers and useful for the elimination of false positives, false negatives of conventional various classifiers, and prognostic evaluation. Moreover, clinician could apply gene therapies to change expression of miRNAs to remedy breast cancer.

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Chapter 67
Dimerization of Chemokine Receptors and its Novel Roles in Drug Discovery

Mingqing Wang, Baosheng Ge and Fang Huang

Abstract G-protein-coupled receptors (GPCRs) comprise the largest family of integral membrane proteins and mediate most of the transmembrane signal transduction. Approximately 50 % of all marketed drugs target GPCRs, which makes this protein family the most important drug targets. Chemokine receptors belong to GPCRs, which were perceived as monomers decades ago. However, recently there are growing evidences indicating that most of GPCRs can form dimers or higher order oligomers. Some chemokine receptors are also found existing as homodimers or heterodimers. A large number of studies have suggested that homodimers or heterodimers may exhibit specific functions, which are different from their monomeric counterparts. Meanwhile, the appearance of dimers with new signaling properties gives new chance in the search for novel drug targets. In this review, we will mainly summarize the current knowledge of the dimerization of chemmokine receptors and its potential roles in drug discovery.

Keywords Chemokine receptor · Dimerization · Drug discovery · GPCRs

67.1 Introduction

G-protein-coupled receptors (GPCRs) comprise the largest family of integral membrane proteins and mediate most of the transmembrane signal transduction [1]. GPCRs are important drug targets and account for about 50 % of all the drugs on the market [2]. Chemokine receptors are a subgroup of the GPCRs superfamily. To date, totally 22 chemokine receptors have been discovered in human genome [3]. Chemokine receptors and their chemokines play an important role in the immune

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defense by directing the migration of leukocytes. In addition to their normal roles, chemokine receptors are involved in many diseases, including inflammatory diseases, cancer, AIDS, and so on [4]. Hence chemokine and receptors are deemed as important drug targets. The first small molecule successfully targeting the chemokine receptor was the CCR5 antagonist, for the prevention of HIV infection, approved by the Federal Drug Administration (FDA) in 2007. Besides, a CXCR4 antagonist was approved by the FDA in 2008 for hematopoietic stem cell mobilization [5]. The existence of dimers or oligomers of GPCRs has been confirmed in the past decades. The structure of chemokine receptor dimers is different from the monomeric form, so it is possible that the dimers function in specific signaling pathways. Moreover, the dimers may take part in ligand binding and distinct signal transduction. The specificity of dimerization may give new opportunity to find novel drugs [6, 7]. Chemokine receptor dimerization and its role in drug discovery are reviewed in this paper.

67.2 Chemokines and Chemokine Receptors

Chemokines are small proteins, which mediate cell migration during inflammation and development. Based on the cysteine (Cys) in the N-terminal domain, chemokines are divided into four classes (CC, CXC, CX3C, XC) [8]. The nomenclature of chemokine is characterized by combination of "L" and an Arabic number, such as CCL1. In the CC class, the two Cys residues are adjacent, and there are 28 known members in human genome. In the CXC class, there is an additional amino acid between the two Cys residues and 17 known CXC chemokines have been discovered till now. The CX3C chemokine has only one known member, with three additional amino acids between the two Cys residues. XC class contains only one cysteine and there are two known members [9, 10].

There are totally 22 known chemokine receptors in human genome [3], and 19 of which works as signaling receptors. The classification of chemokine receptors is based on the ligands which they bind. For example, CC receptors bind CC ligands. The other three chemokine receptors (D6, CCX-CKR, DARC) are atypical receptors, which are unable to directly mediate leukocyte migration. They are thought to regulate chemokine distribution or abundance to control chemokine-derived leukocyte migration. Many chemokines bind multiple receptors and most receptors bind multiple chemokines (Table 67.1). Despite of the pivotal roles in the immune system, chemokine receptors are associated with many diseases, such as atherosclerosis, rheumatoid arthritis, asthma, and cancer (Table 67.1). In addition, CXCR4 and CCR5 are the primary co-receptor for X4 and R5 HIV-1 isolates.

Table 67.1 Chemokine receptor binding their ligands and related to diseases

Chemokine receptor	Binding chemokine	Related to diseases
CXCR1	CXCL6,7,8	Sepsis, atherosclerosis [48]
CXCR2	CXCL1,2,3,5,6,7,8	Sepsis, rheumatoid arthritis [48]
CXCR3	CXCL9,10,11	Transplant, psoriasis, cancer [49]
CXCR4	CXCL12	HIV, cancer [50, 51]
CXCR5	CXCL13	
CXCR6	CXCL16	
CXCR7	CXCL11,12	Cancer [52]
CCR1	CCL3,5,7,8,13,14,15,16,23	Psoriasis, cancer [53]
CCR2	CCL2,7,8,13,16	Diabetes, obesity, cancer [54]
CCR3	CCL5,7,8,11,13,15,16,24,26,28	Allergic diseases, Asthma1 [55]
CCR4	CCL17,22	Asthma, skin disease
CCR5	CCL3,4,5,8,11,14,16	HIV, transplant, cancer [56]
CCR6	CCL20	Asthma [57]
CCR7	CCL19,21	Cancer [58]
CCR8	CCL1	
CCR9	CCL25	Irritable bowel disease [58]
CCR10	CCL27,28	
XCR1	XCL1,2	
CX3CR1	CX3CL1	Atherosclerosis [59]
D6	CCL2,3,4,5,7,8,11,13,14,17,22	
CCX-CKR	CCL19,21,25	
DARC	CCL2,7,8,11,13,14,16,17,18; CXCL1,5,6,7,8,9,11,13	

67.3 Methods to Study GPCR Dimerization

In 1996, Hebert firstly demonstrated that β2-adrenergic receptors could form homodimers by using Co-immunoprecipitation [11]. Since then, many methods have been used to detect the dimerization or higher order oligomerization of many GPCRs [12]. Biochemical techniques are traditional methods to study the dimerization of GPCRs, including SDS–Polyacrylamide Gel Electrophoresis (SDS-PAGE), Cross-linking experiments, Co-immunoprecipitation (Co-IP) and western blot [13]. However, such biochemical techniques can not be used to probe the dimerization of GPCRs in living cells. In the past decade, there was a shift toward using resonance energy transfer techniques to study the dimerization. Applications of bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET) can exhibit advantages over many more traditional techniques because they can detect both intracellular and cell surface expressed dimers in intact living cells [14]. BRET and FRET are based on the nonradioative transfer of energy from a donor molecule to an acceptor molecule in a close distance (less than 10 nm), which have overlapping excitation and emission spectra. Bimolecular fluorescence complementation (BiFC) has also been used to detect the dimerization. BiFC is based on the association between two nonfluorescent fragments of a

Table 67.2 Methods to study GPCR dimerization

Biochemical techniques	Biophysical techniques
SDS-PAGE, Cross-linking experiments, Western blot, Co-immunoprecipitation (Co-IP)	Fluorescence resonance energy transfer (FRET), Bioluminescence resonance energy transfer (BRET), Biomolecular fluorescence complementation (BiFC), Atomic force microscopy (AFM)

fluorescent protein, which could be fused to two GPCRs. If the two GPCRs can interact, then the nonfluorescent fragments can be brought close to each other and thus form a fluorescent protein. In addition, the dimerization and higher order oligomerization of rhodopsin in native retinal disks was studied by using atomic force microscopy [15] (Table 67.2).

67.4 Chemokine Receptor Dimerization and their Functions

GPCRs were considered to exist and function as monomers which can interact with G-proteins in 1:1 stoichiometry. However, in the past decades, the hypothesis was challenged. The growing evidences indicate that GPCRs can physically interact with each other. They can form dimers or even oligomers. In 1998, Jones et al. found the GABAB (a member of class C GPCR) could exist and function as an heterodimer [16]. Both GABABR1 and GABABR2 are nonfunctional when individually expressed in cells, because GABABR1 is retained in the endoplasmatic reticulum (ER), GABABR2 can not bind the ligand [17, 18]. The class C GPCR including sweet and umami taste receptors can function in the form of heterodimer [19, 20].

67.4.1 Chemokine Receptor Homodimerization and Heterodimerization

Some chemokine receptors can exist as homodimers. In the early research, ligands were thought to induce the homodimerization. For example, CXCL12 induced CXCR4 homodimerization [21], while CCL2 and CCL5 could induce the homodimerization of CCR2 and CCR5, respectively [22, 23]. Recent studies suggest that CCR2 [24], CCR5 [25], CXCR1 [26], CXCR2 [27], CXCR4 [28, 29], CXCR7 [30], and DARC [31] can form constitutive homodimerization.

Some chemokine receptors can also form heterodimers. Mellado et al. first reported CCR2/CCR5 heterodimerization as ligand dependent. His study suggested that CCR2/CCR5 heterodimerization was induced by costimulation of CCL2 and CCL5. El-Asmar et al. found CCR5 and CCR2 could heterodimerized