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Research paper

Integrated bioinformatics, computational and experimental methods to discover novel Raf/extracellular-signal regulated kinase (ERK) dual inhibitors against breast cancer cells

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ABSTRACT

Beginning with our previously reported ERK inhibitor BL-EI001, we found Raf1 to be an important regulator in the ERK interactive network, and then we designed and synthesized a novel series of Raf1/ERK dual inhibitors against human breast cancers through integrative computational, synthetic and biological screening methods. Moreover, we found that compound 9d suppressed the proliferation of breast cancer cell lines and induced cellular apoptosis via a mitochondrial pathway with only partial dependence on Raf1 and ERK. Our results suggest that an integrative method including in silico design, chemical synthesis, biological screening and bioinformatics analysis could be an attractive strategy for the discovery of multi-target inhibitors against breast cancer.

1. Introduction

According to 2015 cancer statistics, breast cancer is expected to account for approximately 29% of all new cancer cases among women and is the second most common cancer worldwide after lung cancer [1]. Breast cancers have become one of the most challenging solid tumors to diagnose and treat due to their heterogeneity. Breast cancers occur with a variety of intra-tumor and inter-tumor genetic and epigenetic alterations, so the detection and validation of biomarkers will greatly assist targeted therapy for breast cancer [2]. A number of studies suggest that the Raf/MEK/ERK signaling pathway is hyperactivated in tumors, and targeting the Raf/MEK/ERK pathway plays an important role in the treatment of breast cancer [3–5]. Mitogen-activated protein kinase (MAPK) cascades are related to the regulation of normal cell proliferation, survival and differentiation, while the inhibitor of the extracellular signal-regulated kinase (ERK) MAPK pathway has been used for the treatment of cancer [6–9]. Recently, the use of Raf or MEK inhibitors targeting ERK signaling has shown promising clinical activity in breast cancer treatment [4,10,11]. Therefore, intrinsic and acquired targeting resistance to the Raf/MEK/ERK signaling pathway would be an innovative approach for breast cancer therapy.

In our previous studies, we have designed and synthesized a novel ERK inhibitor, named BL-EI001, that could induce ERK-dependent mitochondrial apoptosis against breast cancer both in vitro and in vivo [12]. Further studies suggest that apoptosis induced by BL-EI001 is independent of the Ras/Raf/MEK pathway in breast cancer cells. These results indicate the complexity of the kinase signaling network. The proteomics analysis of BL-EI001-treated breast cancer cells indicate some potential ERK interactive proteins in breast cancer cells, such as HMGB1, BIRC6 and ATFM2, among others. Many BL-EI001-regulated ERK interactive proteins interact directly or indirectly with another oncogene family, the Raf

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protein family, which is not affected by BL-EI001. In the past few years, some researchers have reported that the inhibition of Raf resulted in potent suppression of different kinds of breast cancers [13–18], so we speculate that the simultaneous inhibition of Raf and ERK might be a feasible therapeutic approach for breast cancer.

To our knowledge, several small molecular Raf inhibitors have been approved by the FDA for cancer therapy, e.g., sorafinib [19,20], vemurafenib [21] and dabrafenib [22], among others. Several B-Raf inhibitors are still in the clinical research stage, such as encorafenib [23], CEP-32496 [24] and RO5126766 [25], among others (Fig. 1A). Their lesser development status means that only a few ERK inhibitors have been registered for clinical trials. The first small molecule ERK inhibitor developed was FR180204 [26], with only modest inhibitory activity. Afterwards, several potent ERK inhibitors, such as VTX-11e [27], SCH772984 [28] and ulixertinib [29], were discovered to inhibit ERK with IC_{50} values at the nanomolar level (Fig. 1B). Most recently, Peng et al. reported the design and synthesis of a novel pan-Raf inhibitor, LY3009120, which bound to both the activated monomer or homodimer of B-Raf and the Ras-dependent heterodimer of B-Raf and Raf1 [30]. Peng et al. suggested that the unique binding modes of the potent Raf1 inhibitor LY3009120 to the RAF kinase domain were responsible for its effectiveness against both B-Raf and Ras mutant cancer cells. It is apparent that a Raf/MEK/ERK pathway inhibitor could be more effective than the current monomeric and dimeric B-RAF inhibitors [31,32]. Therefore, the discovery of novel Raf/ERK dual inhibitors and the validation of their detailed molecular mechanisms are feasible research avenues in breast cancer therapy.

Although several compounds have been reported to possess non-specific multiple inhibitory activities against both Raf and ERK, to our knowledge, there have been few reports of successful Raf1/ERK dual inhibitors for breast cancer therapy. In the current study, we designed and synthesized a series of 4-pyrimidinyl-*N*-arylureas as novel Raf1/ERK dual inhibitors. These compounds potently suppressed the kinase activities of both Raf1 and ERK (Fig. 1C). The IC_{50} values of the most potent compound, 9d, were at the submicromolar level. Herein, we utilized integrated proteomics, computational and experimental methods to discover novel Raf1/ERK dual inhibitors as potential breast cancer therapeutics via inducing apoptosis. The new leads show no apparent toxicity and are suitable for further development for breast cancer therapy.

2. Results and discussion

2.1. Raf is an important regulator in the ERK interactome

Based on the online protein-protein interaction (PPI) databases, we computationally constructed a global human PPI network covering almost all PPIs. We derived physical protein–protein interactions from manually created PPI databases to construct the set

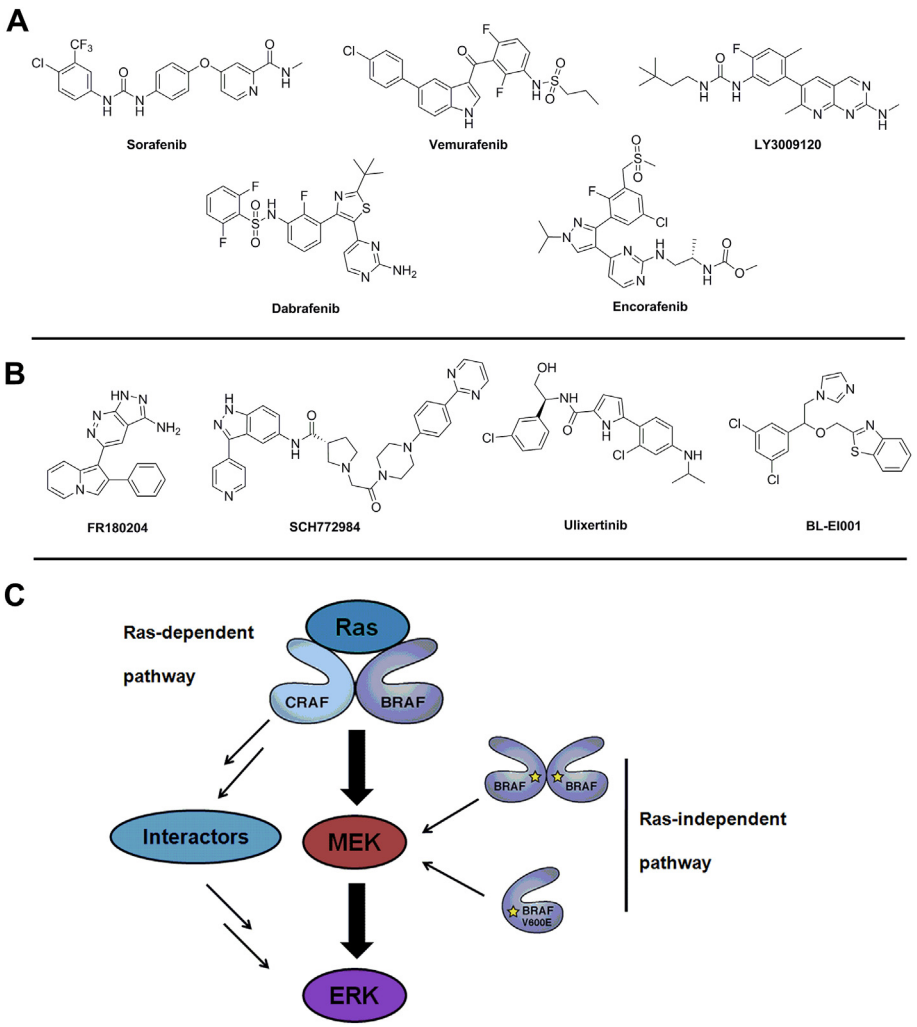

Fig. 1. Chemical structures of selected Raf inhibitors (A), ERK inhibitors (B) and the Ras-dependent or Ras independent mechanism of Raf-ERK pathways (C).

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