Biochimica et Biophysica Acta 1804 (2010) 463–475

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

c-Jun N-terminal kinase (JNK) signaling: Recent advances and challenges

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ARTICLE INFO

Article history:
Received 18 August 2009
Received in revised form 30 October 2009
Accepted 2 November 2009
Available online 10 November 2009

Keywords:
c-Jun N-terminal kinase (JNK)
ATP-competitive inhibitor
ATP non-competitive inhibitor
JNK structure
JNK substrate
Autophagy
microRNA
JNK knockout mouse

ABSTRACT

c-Jun N-terminal kinases (JNKs), first characterized as stress-activated members of the mitogen-activated protein kinase (MAPK) family, have become a focus of inhibitor screening strategies following studies that have shown their critical roles in the development of a number of diseases, such as diabetes, neurodegeneration and liver disease. We discuss recent advances in the discovery and development of ATP-competitive and ATP-noncompetitive JNK inhibitors. Because understanding the modes of actions of these inhibitors and improving their properties will rely on a better understanding of JNK structure, JNK catalytic mechanisms and substrates, recent advances in these areas of JNK biochemistry are also considered. In addition, the use of JNK gene knockout animals is continuing to reveal *in vivo* functions for these kinases, with tissue-specific roles now being dissected with tissue-specific knockouts. These latest advances highlight the many challenges now faced, particularly in the directed targeting of the JNK isoforms in specific tissues.

1. Introduction

The c-Jun N-terminal kinases (JNKs) were first described in the early 1990s [1–4] at a time when the understanding of protein kinase structures and the development of kinase-specific inhibitors were in their relative infancy. Landmark discoveries in the area of JNK research are summarized in Fig. 1. Notably, the earliest discoveries included the identification of the three mammalian JNK genes that specify the JNK isoforms JNK1, JNK2, and JNK3 (also termed stress-activated protein kinase (SAPK)-γ, SAPK-α and SAPK-β, respectively) [4–6].

We have previously reviewed JNK gene knockout phenotypes, particularly noting the interest generated from discoveries of neuroprotection in *JNK3*-/- animals [7,8], and the embryonic lethality of *JNK1*-/-*JNK2*-/- animals [9,10]. Furthermore, non-redundant functions for JNK1 and JNK2 have been revealed in *JNK1*-/- or *JNK2*-/- animals, first in the immune system [11], and then later in a range of disease phenotypes such as diabetes or arthritis (see review [12]). For example, a role for JNK1 in mediating insulin resistance in Type 2 diabetes has been suggested following the observation that *JNK1* deficiency in the genetically obese *ob*/*ob* mice was associated with protection from obesity, hyperglycemia and hyperinsulinemia [13]. Interestingly, the study of *JNK2*-/- animals has implicated that specific isoform in the development of non-obese Type 1 diabetes, through the JNK2 dependent effects on cytokine production [14]. Similarly, the loss of JNK1 or JNK2 expression affected different features of arthritis, with loss of JNK2 showing improved arthritic scores [15].

Alternative splicing further increases the diversity of JNK proteins, however apart from early biochemical studies on these spliceforms [16], their functional significance *in vivo* has remained largely unexplored. Other efforts to understand the biochemical features of these protein kinases have included the structural determination of the different JNK proteins [17–19], together with the discovery of JNK pathway scaffolds such as JNK-interacting protein-1 (JIP1) and related proteins [20–22], and the screening efforts to identify small molecule ATP-competitive inhibitors such as SP600125 [anthra(1,9-cd)pyrazol-6(2*H*)-one] [23]. Therefore, a diverse range of approaches have evaluated the roles for the JNKs in both normal physiology and disease.

The JNKs have continued to attract attention as possible mediators of cell stress responses following their implication as regulators of proapoptotic death signaling events [24–26]. This link between JNK activation and cell death, as revealed by gene knockout and/or the use of inhibitors, has stimulated inhibitor programs to develop JNK inhibitors that can prevent cell death, particularly as neuronal death can underlie both acute and chronic neurodegenerative diseases and

Abbreviations: Ago, Argonaute; DELFIA, Dissociation Enhanced Lathanide Fluoro-Immuno Assay; D-JNKI-1, cell-permeable JIP1-derived JNK inhibitory peptide-1 (D-amino acids); ELISA, enzyme-linked immunosorbent assays; ERK, extracellular signal-regulated kinase (ERK); FITC, fluorescein-isothiocyanate; FRET, Fluorescence Resonance Energy Transfer; GSK, glycogen synthase kinase; JNKs, c-Jun N-terminal kinases; JIP1, JNK-interacting protein-1; L-JNKI-1, cell-permeable JIP1-derived JNK inhibitory peptide-1 (L-amino acids); MAPK, mitogen-activated protein kinase; miRNA, microRNA; MKK, MAPK kinase; Myt1, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase; NMR, nuclear magnetic resonance; PDB, Protein DataBase; PP2C, protein phosphatase 2C; RISC, RNA-induced silencing complex; SAPK, stress-activated protein kinase; Smac, second mitochondria-derived activator of caspases; TAT-TIJP, shorter version of the L-JNKI-1 TAT-linked JIP-based inhibitory peptide; XIAP, X-linked inhibitor of apoptosis

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doi:10.1016/j.bbapap.2009.11.002

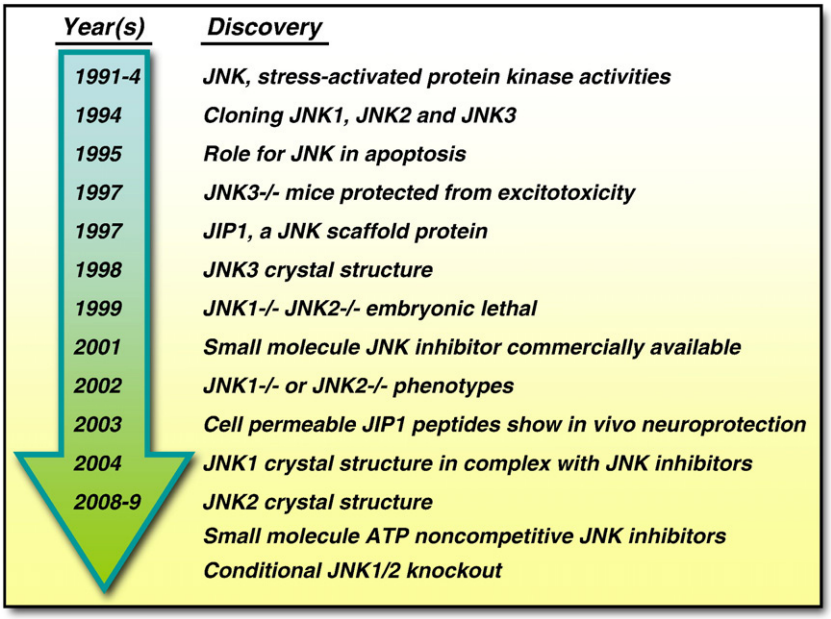

Fig. 1. Landmark discoveries in the area of JNK research. The timeline of significant discoveries from the initial identification of JNKs as stress-activated protein kinases in the early 1990s to the most recent work on JNK2 structure, small molecule ATP-noncompetitive inhibitors and phenotypic characterization of conditional JNK1 and JNK2 gene knockout animals is summarized (refer to Section 1 for a detailed description).

the death of pancreatic β-cells exacerbates the poor control over circulating glucose levels in Type 2 diabetes. While a focus has been ATP-competitive JNK inhibitors, a cell-permeable JNK inhibitory peptide has shown *in vivo* efficacy [27]. Therefore, JNK inhibitors, whether they are ATP-competitive or ATP-noncompetitive in their actions, may provide novel treatments for diseases such as stroke, chronic neurodegeneration, and diabetes.

In this review, we explore the most recent developments in the discovery of JNK inhibitors, and consider recent reports of both ATP-competitive and ATP-noncompetitive JNK inhibitors. Improved inhibitor design and understanding the modes of actions of these inhibitors will rely on a better understanding of JNK structure, JNK catalytic mechanisms and substrates. Therefore, we also present recent advances in these areas of JNK biochemistry. In addition, the use of JNK gene knockout animals is continuing to reveal *in vivo* functions for these kinases, with tissue-specific roles now being dissected with tissue-specific knockouts. These advances highlight the many challenges now faced, including how the directed targeting of the JNK isoforms in specific tissues can be achieved.

2. Recently described ATP-competitive JNK inhibitors—new small molecule drug leads

Conventional inhibitor screening programs have continued to discover small molecule JNK inhibitors. Table 1 summarizes examples of these inhibitors identified since 2007, and the reader is referred to our previous review for small molecule JNK inhibitors described previously [28]. This discovery process has screened proprietary chemical libraries for inhibitors of JNK activity towards substrates such as c-Jun. The level of inhibition has typically been assessed either by a decreased incorporation of ^{32}P from [γ-^{32}P]ATP [29,30] or the decreased phosphorylation of the protein substrate (as determined in antibody-based methods such as enzyme-linked immunosorbent assays (ELISA) or homogeneous time-resolved fluorescence assays [31–33]). Alternatively, a fluorescence anisotropy binding assay has been used to identify compounds that prevent JNK binding of a fluorescently labeled inhibitor, with the expectation that these compounds would act as higher affinity JNK inhibitors [34,35]. Further medicinal chemistry approaches to explore structure–activity relationships have improved inhibitor potency. Examples can be seen in recent publications [29–37]. In addition, some of these approaches have minimized undesirable characteristics such as rapid metabolism in the liver that would compromise inhibitor efficacy *in vivo* [37].

Typically, the identified JNK inhibitors have bound the ATP-binding site and/or targeted the hinge region (see Table 1 for Protein Database (PDB) entries). However, despite the rational approaches in development, some of these subsequent inhibitors have bound JNK in a manner different from the original lead compounds. For example, there was less extensive interaction of 3,5-disubstituted quinolines with the JNK ATP-binding site [36]. This might also permit binding and inhibition of other closely related protein kinases [36]. Also, X-ray crystallography of a novel, potent and selective series of *N*-(3-cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)amide inhibitors revealed a unique binding mode with the 3-cyano substituent forming an H-bond acceptor interaction with the JNK3 hinge region of the ATP-binding site [34]. These studies have highlighted that selectivity profiles must always be re-evaluated during inhibitor development and that the mechanisms of inhibitor binding should be confirmed at all stages of inhibitor development by structural biology approaches.

An improved understanding of JNK structural features may now also drive the development of JNK-specific inhibitors. Most recently, structure–activity relationships of inhibitors combined with structural biology approaches for JNK have described the features for selectivity of aminopyrazole inhibitors towards JNK3 over the related p38 mitogen-activated protein kinase (MAPK) [33]. One potent JNK3 inhibitory compound, SR-3576 (IC_{50} = 7 nM), showed >2800 selectivity over p38 (IC_{50} >20 μM). Its improved selectivity towards JNK3 was attributed to structural features such as its planar pyrazole ring and N-linked phenyl structures that improved interactions in the smaller JNK3 binding site [33].

The modest (~20-fold) selectivity of these aminopyrazole inhibitors towards JNK3 over JNK1 also has suggested that these features may also allow the design of JNK isoform-selective inhibitors [33]. Indeed, with specific functions of the JNK isoforms as revealed in the gene knockout studies (overviewed in Section 1 of this review), the development of inhibitors that show high specificity for a specific JNK isoform will remain a desirable aim of ongoing research in this area. This has been previously highlighted in the review of JNK3 inhibitor