JNK1 and Brain Development

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c-Jun NH2-terminal Kinases (JNKs), also known as Stress-Activated Protein Kinases, are a group of stimuli-response enzyme members of the Mitogen-Activated Protein Kinase (MAPK) family. Proper neuronal development is essential to the correct functioning of brain networks and connections. Due to its biological relevance, brain development is a controlled process that includes multiple regulatory pathways and mechanisms.

JNK1, DCX, SCG10, WDR62, NMDAR

1. Introduction

In mammals, JNK isoforms are codified in three different genes: Jnk1/Mapk8, Jnk2/Mapk9, and Jnk3/Mapk10, which express four splice variants for JNK1 (α1, α2, β1, β2), four variants for JNK2 (α1, α2, β1, β2), and 8 variants for JNK3 (less characterized), all having a molecular weight of either 46 (p46, α1 and β2) or 54 KDa (p54, α2 and β2) with a C-terminal extension; additionally, some splice variants of JNK3 have an N-terminal extension [3–5]. Nonetheless, isoforms exhibit specific patterns of distribution in the organism, e.g., JNK1 and JNK2 are ubiquitous while JNK3 is only expressed in the heart, testicles and brain. Some organelles (such as mitochondria and endoplasmic reticulum) and structures (such as dendritic spines and axon terminals) show special enrichment of JNKs, usually in an isoform-specific manner. These data suggest that every isoform has different regulatory functions, vanquishing the notion of redundancy [3,4].

Different JNKs phosphorylate a wide arrange of nuclear and cytosolic substrates in serine/threonine residues, followed by proline residue, thereby underpinning their role in critical physiological processes that include neuronal functions and immunological responses, together with embryonic and postnatal development [4–6]. This is evidence of the highly differentiated regulatory functions of JNK isoforms [7].

2. The JNKs Signaling Cascade

In the central nervous system (CNS), JNK signaling regulates many cellular processes, ranging from morphogenesis, neuronal pathfinding, axodendritic architecture, neuronal survival, synaptic plasticity and memory formation, as well as energetic and hormonal regulation [11,13–16].

JNKs are prodirected serine/threonine kinases; their activation occurs in a three-step cascade of kinases, starting with the Kinase Kinase (MAPKKKs). MAPKKKs phosphorylate and activate members of the MAPK Kinase family

(MAPKK), such as MAPK/ERK Kinase 4 (MKK4) and MAPK/ERK Kinase 7 (MKK7) which, in turn, activate MAPKs (JNK1/SAPK1, JNK2/SAPK2, and JNK3/SAPK3) in conserved dual phosphorylation sites of threonine and tyrosine (TPY motif sequence). Scaffold JNK-interacting proteins (JIP) facilitate the sequential phosphorylation cascade and MAPK phosphatases (MKPs) deactivate this pathway [5,17–19].

The protein structure of JNK has two globular domains which are characterized by a short N-terminal and large C-terminal peptide chain connected by a flexible domain, i.e., the catalytic site present in a deep cleft domain on the interface of lobules. Their mechanism of the action comprises multiple steps, starting with their acquisition of an open conformation in the absence of ATP, and a closed conformation when activated and bound with the substrate [20]. Several motifs are necessary to regulate the enzymatic reaction: the activation domain (A-loop) contains the TPY sequence, and a conserved G-loop (Glycine-Rich) motif is necessary to close the ATP-binding domain. The HRD motif is involved in the reaction mechanics; meanwhile, the DRG sequence is critical for conformational changing in the reaction [20,21]. JNKs also contain D-recruiting site (DRS). This sequence is necessary for interactions with their cognate sequence D-motif, found in substrates or scaffold-protein-like JIP [21]. In general, the catalytic site is conserved (See protein alignment for the main JNK isoform, Supplemental Material Table S1)

Disruptions of physiological signaling of the JNKs may lead to adverse outcomes, both when the pathway is down- or up- regulated. For example, there is evidence that links dysregulations in the signaling of the JNK pathway with neurodegenerative diseases including Parkinson's [22], Alzheimer's [23] and Huntington's diseases [24,25], polyglutamine diseases [26] and auditory hair cell degeneration [27]. Moreover, defects in JNK activation, with a key role in axonal growth, explain the neurodevelopmental deficits that appear in lysosomal storage diseases [1,3,4,28]. In line with this, genetic studies in patients have associated truncated mutation of JNK3, induced by translocation in chromosome 4 and Y, with severe developmental epilepsy and intellectual disability. This mutation prompts the of loss-of-function of the JNK signaling pathway, preventing the phosphorylation of the classical JNK target c-Jun and other substrates such as PSD95 [29,30]. Moreover, other studies have shown that changes in physiological signaling of the MKK7/JNK cascade may underlie the neurochemical alterations which are associated with schizophrenic symptoms [31]. Genetic association studies have linked schizophrenia with the microduplication of several genes, including TAO2 kinase, an upstream activator of the JNKs [32]. Finally, mice knockout of *Jnk1*, or mice treated with an engineered retrovirus that exclusively inhibited JNK1 in adult-born granule cells, displayed an increase in immature neurons in the dentate gyrus of the hippocampus, an effect that has been correlated with a reduction in depressive-like behaviors [33]. Our results provide evidence that a lack of *Jnk1* in mice offers neuroprotection against excitability and the cognitive impairments induced with high fat diets (HFD) [34].

3. Cytosolic Substrates of JNK in Developing Neurons

Over the years, biochemical studies have allowed us to identify multiple substrates to JNKs located in the cytoplasm or nucleus. In the nucleus, JNKs regulate multiple transcription factors which are responsible for gene expression, e.g., c-Jun, activating transcription factor 2 (ATF2), E26 transformation-specific-like 1 (Elk-1), p53, nuclear factor of activated t-cells 4 (NFAT4) and other chromatin modifiers [3]. However, bioinformatic studies of D-motifs have revealed that multiple JNK substrates may comprise cytoskeletal proteins such as actin-binding

proteins, microtubule-binding proteins (MAPs), motors proteins, centrosomal proteins, basal bodies and others involved in primary cilium biogenesis [5]. Functional analyses have confirmed that JNKs phosphorylate several MAPs, including Microtubule-Associated Protein 1B (MAP1B), Microtubule-Associated Protein 2 (MAP2), Microtubule Associated Protein Tau [35], Doublecortin (DCX) [36] and superior cervical ganglion protein 10 (SCG10; also known as Stathmin 2 (STMN2) [37]. In fact, some estimates link JNK signaling with at least 60% of the total phosphorylation occurring in the growth cone during brain development, affecting substrates like the Growth Associated Protein 43 (GAP43), MAP1B, SCG10, RUN FYVE Domain Containing 3 (RUFY3) and Roundabout Guidance Receptor 2 (ROBO2) [38].

Furthermore, single neurites accumulate mRNAs of elements belonging to the JNK pathway, such as *Jnk1, Mkk4, Mkk7*, *Dual Leucine Zipper Kinase (Dlk),* a novel *SCG10*-like protein (*Sclip*), *Map2, Map1b*, and Scaffold JNK-interacting protein family members, i.e., *JipP4/JlpP* and *Jip3/JSap* [39]. Local translation rates suggest a high turnover of JNK signaling components in neurites.

4. JNKs Have Specific Functions. JNK1 is Essential for Proper CNS Development

The different JNKs isoforms have specific functions and respond differently, according to the type of stimulus, duration, cell or subcellular compartment [6,40]. In the CNS, JNK1 activity is the foremost contributor to total JNK activity; it takes part in many processes, including in the maintenance of axonal tracks during development or the control of cognitive function [4,28,41].

The use of knockout (KO) mice for the JNK1 isoform has given rise to reports that this isoform is essential for proper CNS development. It was described that the absence of a single isoform is not lethal in transgenic knockout mice, but that double null mutant embryos for JNK1 and JNK2 ($Jnk1^{-/-}Jnk2^{-/-}$) die between embryonic day E11 and 12. These mice fail to close the neural tube due to a decrease in apoptosis rate in the hindbrain neuroepithelium and an increase in the forebrain correlated with widespread caspase activation. Furthermore, $Jnk1^{-/-}Jnk2^{-/+}$ mice present retinal coloboma (failure in fissure closure) and show severe defects in the lens at E18. These mice die after birth due to severe deficiencies in their development. These JNK isoforms play a different role in apoptosis, depending on the brain region where they are located, suggesting that JNK1 and JNK2 have both proapoptotic and apoptotic functions. Moreover, the absence of lethality in $Jnk1^{-/-}/Jnk3^{-/-}$ or $Jnk2^{-/-}/Jnk3^{-/-}$ embryos, together with the lack of apoptotic alterations in these double mutant mice, suggests that JNK1 and JNK2, but not JNK3, have a redundant role in the regulation of apoptosis in specific brain areas during brain embryogenesis [42,43].

$Jnk1^{-/-}Jnk2^{-/+}$ mice present retinal coloboma (failure in fissure closure) and show severe defects in the lens at E18. These mice die after birth due to severe deficiencies in their development. Importantly, while a partial expression of JNK2 is not enough to compensate for a lack of JNK1, monoallelic expression of *Jnk1* in $Jnk1^{-/+}Jnk2^{-/-}$ knockout mice leads to a nonobvious altered phenotype which allows the animals to survive [44]. Nonetheless, mice null for the *Jnk1* gene have the anterior commissure disrupted and exhibit alterations in the organization of cortical area,

with no clear consensus regarding the consequences of this. Chang et al. reported that these mice showed growing degeneration of axonal and dendritic processes (visible in 8-month-old animals), jointly with progressive learning impairment and motor defects [41,45,46]. Our research group observed no behavioral alterations when testing 9-month-old *Jnk1*$^{-/-}$ animals using the novel object recognition test [34].

5. JNKs Regulate Programmed Cell Death During Brain Development

Neuronal death is a highly conserved and relevant process in brain development that is controlled by JNKs. It plays a role in the control of synaptic connections and morphogenesis, e.g., in the elimination of defective and abnormal cells [47]. As Kuan et al. reported, the JNK signaling pathway controls caspase activity in brain embryonic regions [43]. However, the mechanism of JNK activity in programmed cell death during development remains under study. In this line, our results showed a different number of immature neurons in the subgranular zone of the dentate gyrus of the adult hippocampus in knockouts (KOs) for JNK compared with wild type (WT) [14]. However, the mechanism of JNK activity in programmed cell death during development remains unclear. In this line, our results showed a different number of immature neurons in the subgranular zone of the dentate gyrus of the adult hippocampus in knockouts (KOs) for JNK, compared with wild type (WT) [47]. Since AGO is highly conserved across both the animal and plant kingdom, the role of the AGO1-TAK1-JNK pathway in programmed cell death in brain in mammalian development must be analyzed. In addition, it would be useful to identify the role of canonical Wnt pathway signaling in programmed cell death in the CNS in mammals, because Wnt signaling, in collaboration with the JNK pathway, promotes dorsal closure and ventral patterning during *Drosophila* embryogenesis [48].