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Cross-reactivities of mammalian MAPKs antibodies in rotifer and copepod: Application in mechanistic studies in aquatic ecotoxicology

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ABSTRACT

The mitogen-activated protein kinases (MAPKs) family is known to mediate various biological processes in response to diverse environmental pollutants. Although MAPKs are well characterized and studied in vertebrates, in invertebrates the cross-reactivities of MAPKs antibodies were not clearly known in response to environmental pollutants due to limited information of antibody epitopes with material resources for invertebrates. In this paper, we performed phylogenetic analysis of MAPKs genes in the marine rotifer *Brachionus koreanus* and the copepods *Paracyclopina nana* and *Tigriopus japonicus*. Also in rotifer and copepods, several studies of Western blot of MAPK signaling pathways were shown in response to environmental pollutants, including multi-walled carbon nanotubes (MWCNTs), water-accommodated fractions (WAFs) of crude oil, and microplastics. This paper will provide a better understanding of the underlying mechanistic scenario in terms of cross-reactivities of mammalian antibodies in rotifer and copepod.

1. Introduction

Signal transductions are a pathway that ultimately triggers an intracellular biochemical event in response to extracellular stimuli. Among various signal transduction pathways, mitogen-activated protein kinases (MAPKs) are considered as one of the most important pathways due to their broad range of involvement with crucial cellular processes including cell proliferation, differentiation, survival, death, and transformation (McCubrey et al., 2006; Torii et al., 2006; Dhillon et al., 2007). The highly conserved MAPKs, serine/threonine kinases comprises three subgroups namely, extracellular signal-regulated kinases (ERK), c-Jun. amino-terminal kinases (JNK), and p38 mitogen-activated protein kinases (p38) (Widmann et al., 1999). These MAPKs are activated by phosphorylation at specific sites (e.g. serine/threonine) in their amino acid sequences which, in turn, are activated by upstream MAPK kinases. Each activated MAPKs play a central role in signal cascades by mediating target gene expression via modification of their corresponding transcription factor activity in response to various stimuli such as growth factor, cytokine, oxidative stress, heat shock, and irradiation (Widmann et al., 1999; Kyriakis and Avruch, 2001; Johnson and Lapadat, 2002).

Here, we provide current status of studying MAPKs signaling pathways in invertebrate in terms of ecotoxicology based on the below reasons; MAPKs pathways are (1) involved in various stress-responsible pathways, (2) universally conserved among animal taxa, and (3) appropriate for comparative studies over vertebrates. This paper provides key biochemical information using Western blot on the mode-of-action of environmental pollutants in rotifer and copepod and would be helpful for establishing rotifer and copepod as a model species for in-depth molecular mechanistic studies.

2. Rotifer and copepod for molecular ecotoxicology studies

Invertebrates make up 95% of all animal species on Earth (Myers et al., 2000) and play important roles in the ecosystem. Among invertebrates, rotifer and copepod take a central trophic level in the aquatic ecosystem by transferring energy from producers to consumers (Snell and Janssen, 1995; Dahms et al., 2011; Raisuddin et al., 2007). Rotifer and copepod also has many advantages as experimental species such as their small size (~150 μm for rotifer and ~600 to 1000 μm for copepod), short generation cycle (~24 h for rotifer and ~2 weeks for copepod), simple structure, high fecundity, and easy laboratory maintenance.

Moreover, some of invertebrates are available for genome information (http:// arthropodgenomes.org/wiki/i5K), improving them as a model species in many areas of molecular biology. For examples, the fruit fly *Drosophila melanogaster*, one of the most well established

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invertebrate model species, is available for its genome information that enable to make major contributions in understanding development and embryogenesis and also revealed that most of *Drosophila* genes and pathways are generally conserved compared to higher animals (reviewed by Beckingham et al., 2005). The genome information of the nematode *Caenorhabditis elegans* has been identified that up to 80% of their genes are homologue of human genes, whereas 12 signal transduction pathways of 17 known pathways are conserved between *C. elegans* and human (Kaletta and Hengartner, 2006). As a marine invertebrate, the genome database of the rotifer *Brachionus koreanus* and the copepods *Tigriopus japonicus* (196 Mb) and *Paracyclopina nana* (85 Mb) have recently constructed (unpublished data) and are being used for understanding the underlying molecular mechanisms in response to marine pollutants (Dahms et al., 2016; Lee et al., 2015). Thus, the genome database of invertebrates would contribute to establish the concept of toxicity and the corresponding physiological mechanisms and also provides new insight in to the understanding biological process and cellular responses in invertebrates. Based on these advantages of invertebrates, they have increased attention as a model species in the field of molecular ecotoxicology.

3. MAPK pathways in rotifer and copepod

3.1. Gene analysis of MAPKs in rotifer and copepod

MAPKs in mammals are consisted of 14 genes; conventional MAPKs and atypical MAPKs. Conventional MAPKs are comprised of ERKs (ERK1/2) JNKs (JNK1/2/3), p38 (α, β, γ/δ), and ERK5, whereas ERKs (ERK3/4, ERK7/8) and Nemo-like kinase (NLK) are belong to atypical MAPKs (Cargnello and Roux, 2011).

In invertebrates, 5 and 7 MAPK genes were identified in the genome of *D. melanogaster* and *C. elegans*, respectively (Table 1). *Rolled* in *D. melanogaster* and *mpk-1* in *C. elegans*, *basket* in *D. melanogaster* and *jnk-1*, *kgb-1*, and *kgb-2* in *C. elegans*, and *Dp38a*, *Dp38b*, and *Dp38c* in *D. melanogaster* and *pmk-1*, *pmk-2*, and *pmk-3* in *D. melanogaster* were identified as homologous to mammalian *ERKs*, *JNKs*, and *p38s*, respectively. In addition to *D. melanogaster* and *C. elegans*, in rotifer and copepod, we firstly report *MAPKs* genes. In the genome of the copepods *T. japonicus* and *P. nana*, four *MAPK* genes were identified. Of four *MAPK* genes, two *Erks*, one *Jnk*, and one *p38* were identified the homologue relationship with human and other model invertebrates. In the rotifer *B. koreanus*, two genes were identified as homologue to *ERK*, one gene as *JNK*, and two genes as *p38* (Table 1). Here, we followed the guideline from HUGO Gene Nomenclature Committee for gene nomenclatures of identified genes as shown in *MAPKs* in *B. koreanus*, *P. nana*, and *T. japonicus*.

MAPK pathways are highly conserved over species and examined their activation in MAPK pathways in response to various stimuli in several invertebrates, as the conservation of MAPK genes is likely responded to similar ways as shown in mammalian system on their function. In the genomes of the rotifer *B. koreanus* and the copepds *P. nana*, and *T. japonicus*, putative *MAPK* genes were subjected into phylogenetic analysis with other species including *C. elegans*, *D. melanogaster*, and *Homo sapiens* (Fig. 1). Detailed descriptions about each subfamily of MAPK are as below. (See Fig. 2.)

3.1.1. ERK

In the worm *C. elegans*, homologue of *ERK* to *H. sapiens* was identified as *mpk-1* (Lackner et al., 1994; Wu and Han, 1994), whereas it was identified as *rolled* in *D. melanogaster* (Biggs and Zipursky, 1992; Biggs et al., 1994). In of the rotifer *B. koreanus* and the copepods *P, nana* and *T. japonicus*, two *Erk* genes (*Erk-1/2*) were identified in the genome. The length of complete amino acid sequences of *Erk-1* and *Erk-2* in the rotifer *B. koreanus* and the copepods *P, nana* and *T. japonicus* were 387 and 387 amino acids (aa), 403 and 565 aa, and 408 and 415 aa, respectively. Also the protein kinase domain and phosphorylation site (threonine–glutamic acid–tyrosine residue), which is responsible for conserved function and activation, was highly conserved among these species used in our analysis (Fig. S1). The identity and similarity of *Erk* genes were ranged in 69.65 to 76.66% and 81.22 to 83.72% in *B. koreanus*, 60.15 to 84.44% and 50 to 85.21% in *P. nana*, and 60.42 to 82.22% and 75.58 to 82.72% in *T. japonicus* respectively, compared to *ERK* in *H. sapiens* (Table S1). These scores are similar to *C. elegans* (70.71 to 77.22% for identity and 76.07 to 77.07% for similarity) and *D. melanogaster* (75.53 to 78.88% for identity and 86.71 to 87.20% for similarity), suggesting that *Erk* genes in *B. koreanus*, *P. nana*, and *T. japonicus* are highly conserved with *H. sapiens* as shown in those of *C. elegance* and *D. melanogaster*.

3.1.2. JNK

In invertebrates, homologue to human *JNK* has been characterized as *basket* in *D. melanogaster* (Riesgo-Escovar et al. 1996) and as *jnk-1*, *kgb-1*, and *kgb-2* in *C. elegans* (Kawasaki et al., 1999; Smith et al., 2002). In the rotifer *B. koreanus* and the copepods *P. nana* and *T. japonicus*, one *Jnk* gene was identified in each of their genome and was clustered with other *JNK* homologues by phylogenetic analysis (Fig. 1), while they are evolutionarily conserved domains for protein kinase domain and phosphorylation sites (Fig. S2). The scores for identity and similarity between *Jnks* in rotifer and copepod and *JNKs* in human were 67.01 to 68.83% and 67.83 to 73.16% for the rotifer *B. koreanus*, 67.3 to 71.12% and 74.81 to 80.14% in the copepod *P. nana*, and 65.64 to 69.78% and 71.13 to 76.28% in the copepod *T. japonicus* (Table S2), respectively. These scores were similar or even higher compared to *C. elegans* (45.89 to 58.78% for identity and 52.94 to 58.45% for similarity) and *D. melanogaster* (73.11 to 76.34% for identity and 70.95 to 77.57% for similarity). In rotifer and copepod, the conservation of these genes suggests that they are also highly conserved in functions, as already reported in *C. elegans* and *D. melanogaster* (Widmann et al., 1999).

3.1.3. p38

In the genome of the copepods *P. nana* and *T. japonicus*, there was one gene, showing homologous relationship with human *p38* gene, while two homologue genes were found in the rotifer *B. koreanus*. All these *p38* genes were clustered with other genes homologue to human *p38* by phylogenetic analysis (Fig. 1). As for other MAPK subfamilies, rotifer and copepod protein kinase domain and phosphorylation

Table 1
Information about the MAPK genes in *H. sapiens* and invertebrates.

H. sapiens	*D. melanogaster*	*C. elegans*	*B. koreanus*	*P. nana*	*T. japonicus*
ERK1 (NP_002737)	Rolled (NP_001015122)	mpk-1 (NP_001022584)	Erk-1 (submitted)	Erk-1 (submitted)	Erk-1 (submitted)
ERK2 (NP_620407)			Erk-2 (submitted)	Erk-2 (submitted)	Erk-2 (submitted)
JNK1 (NP_620637)	Basket (NP_723541)	jnk-1 (NP_001021270)	Jnk-1 (submitted)	Jnk-1 (submitted)	Jnk-1 (submitted)
JNK2 (NP_002743)		kgb-1 (NP_499922)			
JNK3 (NP_001304998)		kgb-2 (NP_500384)			
p38α (NP_620581)	Dp38a (NP_477163)	pmk-1 (NP_501365)	p38–1 (submitted)	p38–1 (All16579)	p38–1 (AGS12617)
p38β (NP_002742)	Dp38b (NP_477361)	pmk-2 (NP_741457)	p38–2 (submitted)		
p38γ (NP_002960)	Dp38c (NP_996277)	pmk-3 (NP_501363)			
p38δ (NP_002745)					

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