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Neutralization of viral infectivity by zebrafish c-reactive protein isoforms

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ABSTRACT

This work explores the unexpected *in vivo* and *in vitro* anti-viral functions of the seven c-reactive protein (*crp1-7*) genes of zebrafish (*Danio rerio*). First results showed heterogeneous *crp1-7* transcript levels in healthy wild-type zebrafish tissues and organs and how those levels heterogeneously changed not only after bacterial but also after viral infections, including those in adaptive immunity-deficient *rag1*$^{-/-}$ mutants. As shown by microarray hybridization and proteomic techniques, *crp2*/CRP2 and *crp5*/CRP5 transcripts/proteins were among the most modulated during *in vivo* viral infection situations including the highest responses in the absence of adaptive immunity. In contrast *crp1*/CRP1/and *crp7*/CRP7 very often remained unmodulated. All evidences suggested that zebrafish *crp2-6*/CRP2-6 may have *in vivo* anti-viral activities in addition to their well known anti-bacterial and/or physiological functions in mammalians. Confirming those expectations, *in vitro* neutralization and *in vivo* protection against spring viremia carp virus (SVCV) infections were demonstrated by *crp2-6*/CRP2-6 using *crp1-7* transfected and/or CRP1-7-enriched supernatant-treated fish cells and *crp2-5*-injected one-cell stage embryo eggs, respectively. All these findings discovered a *crp1-7*/CRP1-7 primitive anti-viral functional diversity.These findings may help to study similar functions on the one-gene-coded human CRP, which is widely used as a clinical biomarker for bacterial infections, tissue inflammation and coronary heart diseases.

1. Introduction

Widely used as a general biomarker for bacterial infection and inflammation during many decades, circulating human pentameric CRP (pCRP) has been found recently within atherosclerotic lesions and might be used as a new biomarker for cardiovascular diseases (Shrivastava et al., 2015). Correlation between infections and cardiovascular heart diseases has been demonstrated not only for bacteria but also for several viral infections (Adinolfi et al., 2014; McKibben et al., 2016; Voulgaris and Sevastianos, 2016; Wu et al., 2016). Furthermore, although pCRP was initially discovered during acute-phase responses to bacterial infections increasing their circulating levels from < 10 to > 500 mg/l, intermediate concentrations of 10–50 mg/l were also detected during viral infections (Shah et al., 2015), suggesting that pCRP may have also anti-viral function(s). At this respect, viral infections induce human interferon alpha that represses the *crp* promoter, suggesting also pCRP antiviral effects (Enocsson et al., 2009). Nevertheless and despite pCRP being one of the most investigated risk biomarker molecule in the human cardiovascular field, and an important component of the anti-bacterial innate responses (Vilahur and Badimon, 2015), to our knowledge, there is no evidence yet that pCRP has any antiviral function.

In contrast to the one-gene *crp* of humans, zebrafish (*Danio rerio*) has 7 *crp* genes, from *crp1* to *crp7* (here simplified as *crp1-7* or CRP1-7 for their derived proteins). Amino acid variations among CRP1-7 proteins were mostly found in both their Ca^{++}-dependent phospholipid-binding pocket and conformational-domain sequences (Bello et al., 2017; Chen et al., 2015; Falco et al., 2012). By offering an easy-to-screen *in vivo* system for novel therapeutic molecules, zebrafish supplies a suitable model to explore CRP lipid-binding properties and conformation-dependent functionalities related to cardiovascular heart diseases including viral infections. Zebrafish is a well known model for heart development and function (Genge et al., 2016; Lu et al., 2016; Pitto et al., 2011) and a well known target for several fish rhabdoviruses (Encinas et al., 2013; Estepa and Coll, 2015a; Garcia-Valtanen et al., 2017; Varela et al., 2016). In this context, we have first explored *crp1-7*/CRP1-7 transcript/protein levels during several zebrafish viral infection situations and then designed several *in vitro*/*in vivo* strategies to explore

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