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Comparative endocrinology of leptin: Assessing function in a phylogenetic context

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ABSTRACT

As we approach the end of two decades of leptin research, the comparative biology of leptin is just beginning. We now have several leptin orthologs described from nearly every major clade among vertebrates, and are moving beyond gene descriptions to functional studies. Even at this early stage, it is clear that non-mammals display clear functional similarities and differences with their better-studied mammalian counterparts. This review assesses what we know about leptin function in mammals and non-mammals, and gives examples of how these data can inform leptin biology in humans.

1. Introduction

Nine years have passed since the first leptin sequence was identified in a non-mammalian vertebrate (*Takifugu rubripes*; Kurokawa et al., 2005). Due largely to advances in genomic technology, leptin and leptin receptor genes now have been cloned from all the major vertebrate classes, with the possible exception of Aves (Fig. 1). Although non-mammal leptin studies still comprise ~1% of all leptin studies (Web of Science returns ~29,000 leptin studies (non-reviews or editorials); <300 of those focus on non-mammals), there is now a sufficient body of literature to approach the question of whether leptin function is conserved among vertebrates (Fig. 2). Comparative study of leptins has the goal of not only solving comparative questions, but also clarifying our understanding of leptin function in mammals (by uncovering the origin of leptin function). Thus in this review we describe recent advances of leptin biology in both comparative and biomedical contexts.

2. Leptin's tertiary structure is conserved among vertebrates

One aspect that both aided and hindered leptin research in non-mammalian systems was its structure. Early studies assumed conserved primary structure between mammalian and fish leptins, and thus interpreted fish immunoreactivity with anti-mouse leptin antibodies as evidence of leptin expression (e.g. Johnson et al., 2000). However, traditional redundant-primer strategies were unsuccessful at amplifying a fish leptin for over a decade. Kurokawa's insight of searching for leptin in a conserved pattern of genes, or gene synteny, finally resulted in a true non-mammal leptin gene from a fish genome (Kurokawa et al., 2005). With this breakthrough, the non-intuitive idea that tertiary structure was conserved even though primary sequence was not gained support with each new leptin clone (Crespi and Denver, 2006; Denver et al., 2011; Gorissen et al., 2009; He et al., 2013; Prokop et al., 2012). Leptin orthologues are now described for all major classes of vertebrates (with the exception of birds (Fig. 1)), and all are predicted to have a very similar, class I helical cytokine tertiary structure. It is important to note, however, that crystal structure has been determined for only a single leptin, and that structure was for a leptin modified to enhance crystal formation (human e-100 leptin; Zhang et al., 1997). All non-mammal leptin structures (and most mammal leptin structures) are inferred by modeling algorithms; an empirical determination of a non-mammal leptin structure is needed to validate the computationally derived models.

3. Basal vertebrates express multiple leptin orthologs

All mammals (e.g. Ball et al., 2013; Clarke et al., 2001; Comuzzie et al., 1997; Zhang et al., 1994) and amphibians (Boswell et al.,

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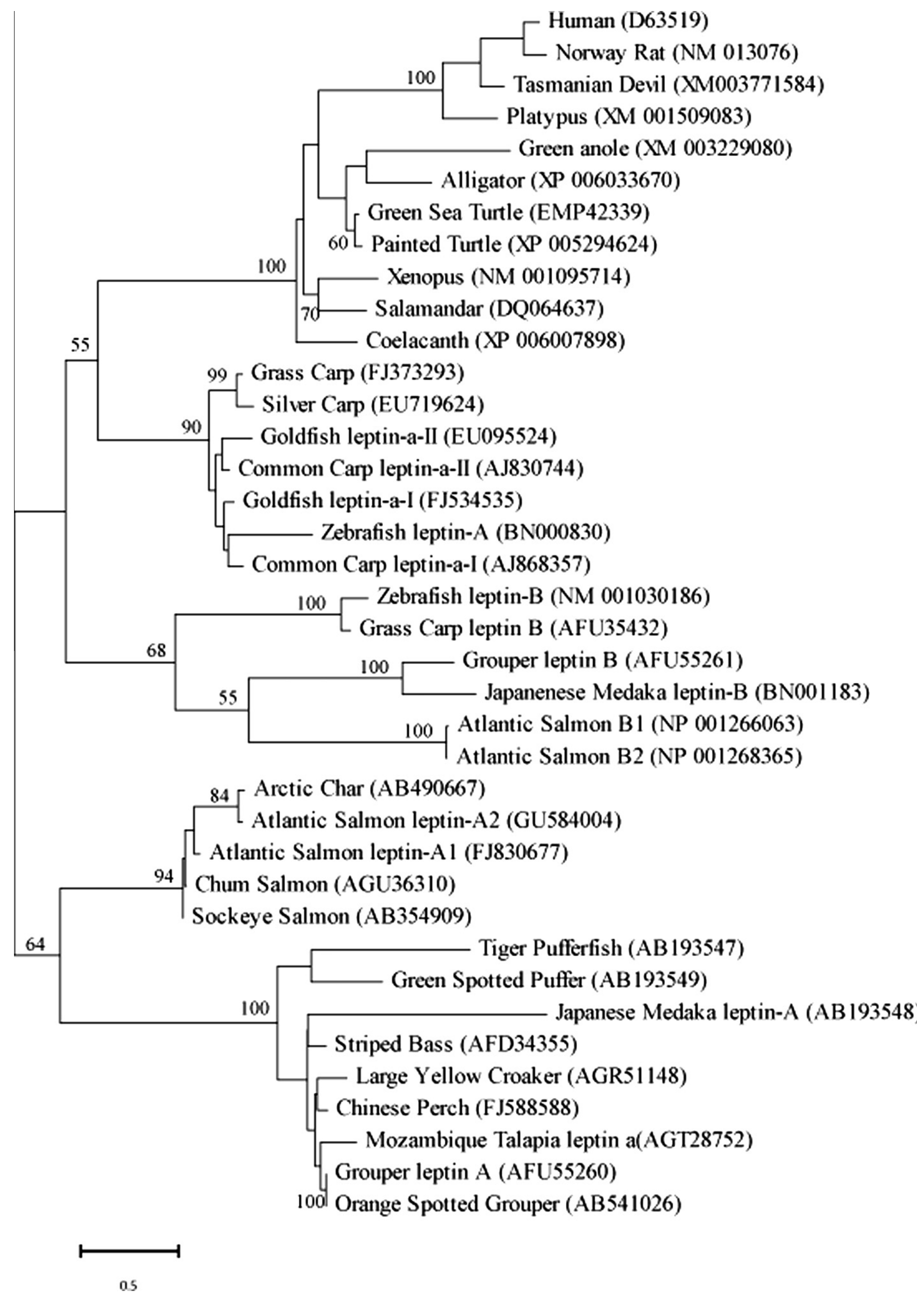

Fig. 1. Phylogenetic tree of 29 boney fish and representative tetrapod leptins. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al., 1992) as conducted in MEGA5 (Tamura et al., 2011). Numbers at nodes represent percentage of 100 bootstrap replicates. Notes with no number indicate bootstrap support of less than 50%. Inferred leptin amino acid sequences were manually aligned in MEGA5 informed by protein structural homologies. GenBank accession numbers in parentheses represent protein accessions.

2006; Crespi and Denver, 2006) express a single ortholog of leptin (*lep*). Two *lep* orthologues are present in the green anole (lizard) genome, but only one may be expressed (Denver et al., 2011). The presence of multiple orthologs within a genome is generally attributed to genome and/or gene duplication (Gorissen et al., 2009; Kurokawa and Murashita, 2009; Ronnestad et al., 2010). Fish leptins are, by far, the best-studied among non-mammal leptins (Fig. 2). Initially our group proposed that all fishes express two *lep* paralogs (reviewed by Copeland et al., 2011), with the possible exception of *Fugu rubripes* (Kurokawa et al., 2005). Now, more recent work indicates that some advanced fishes (including *Fugu* and other Percomorphs) lost the second *lep B* ortholog (striped bass *Morone saxatilis* and stickleback *Gasterosteus aculeatus*; Won et al., 2012, and chinese perch *Siniperca chuatsi*; He et al., 2013). The scenario of vertebrates expressing a single paralog after the Percomorph split (advanced by Won et al., 2012) is contradicted by two leptin paralogs in more-derived groups (orange-spotted grouper *Epinephelus coioides* (Zhang et al., 2013) and green anole (Denver et al., 2011)).

Leptin orthologs now are identified for many different vertebrate taxa separated by considerable evolutionary time (Fig. 1). Given the caveat that the number of orthologs per species is often revised (up) as each genome is analyzed (e.g. initial estimates in salmonids did not recognize a leptin B; Angotzi et al., 2013), we can state that at least some advanced fish taxa express a single leptin ortholog (e.g. *Takifugu*, given that its genome was among the first to be completed and the extent to which it is annotated) and some advanced fish taxa express two leptins (grouper; Zhang

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