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Targeting individual calpain isoforms for neuroprotection

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Calpain overactivation is implicated in the neurodegeneration resulting from a wide range of neurologic disorders and neurodegenerative conditions including stroke, traumatic brain and spinal cord injury, and Alzheimer's disease (Bever and Neumar, 2008; Saatman et al., 2010; Vosler et al., 2008). The best-characterized calpains, and those predominant in the CNS, are the ubiquitous m- and μ -calpains. A major difference between these two isoforms is the Ca^{2+} requirement of the purified enzymes, with m-calpain requiring millimolar Ca^{2+} for activation as compared to micromolar Ca^{2+} for μ -calpain (Dayton, 1982). Other calpains present in the CNS include 3, 5, 10, and 12 (Konig et al., 2003; Ma et al., 2001; Shin et al., 2004; Waghray et al., 2004), although their roles and relative activities are largely unknown.

While calpain overactivation contributes to neurodegeneration, calpains also serve essential physiological roles including signal transduction, cell migration, membrane fusion, and cell differentiation. Thus, the challenge is to inhibit the pathological consequences of calpain overactivation but preserve physiologic aspects of calpain function. One approach is to identify calpain isoforms with a prominent pathological role, as opposed to those which are essential for physiological calpain functions. The high Ca^{2+} levels required to activate m-calpain would presumably only be encountered under pathologic conditions; therefore m-calpain has been hypothesized to represent a pathologic isoform. However, m-calpain may be activated by much lower Ca^{2+} levels in vivo or by non- Ca^{2+} mechanisms including trophic factors (Goll et al., 2003; Zadrav et al., 2010). Alternatively, support for μ -calpain as a pathologic isoform was based on its distribution and that of its cleaved substrate, α II-spectrin, following CNS insults (Grynszpan et al., 1997; Hamakubo et al., 1986; Roberts-Lewis et al., 1994; Siman et al., 1985). However, the substrates of the two calpain isoforms are highly similar, at least in vitro, and small molecule calpain inhibitors inhibit both isoforms with similar potency (Goll et al., 2003; Wang and Yuen, 1999). As a result, it has been difficult to identify distinct roles for these two ubiquitous calpain isoforms.

With the recent advances in knockout and siRNA methodology, distinct roles for individual calpain isoforms have begun to emerge (Croall and Ersfeld, 2007). For the m- and μ -calpains, knockout and knockdown strategies have targeted the unique large subunits, calpain 1 for μ -calpain or calpain 2 for m-calpain, or the shared calpain small subunit 1 also referred to as calpain 4 (Goll et al., 2003). The embryonic lethality of calpain 2 null mice suggests an essential role for m-calpain, and is due to impaired implantation at a pre-blastocyst stage (Dutt et al., 2006). Although calpain 1 is present in the embryonic stem cells, it cannot compensate for the loss of calpain 2 during early development. In contrast,

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the viability and minimal phenotype of calpain 1 null mice (Azam et al., 2001) demonstrates that μ -calpain is not essential. Based on siRNA studies, m-calpain also appears to have a more prominent role in cell adhesion and motility (Franco et al., 2004; Franco and Huttenlocher, 2005).

The recent report from Bevers et al. (2010) is the first to identify neuropathologic roles of individual calpain isoforms in vivo. Knockdown of calpain 1/ μ -calpain attenuated spectrin proteolysis by calpain following transient forebrain ischemia, prevented the loss of hippocampal CA1 neurons, and preserved electrophysiologic function. Knockdown of calpain 2/m-calpain was less effective in reducing calpain activity and improving the survival of hippocampal CA1 pyramidal neurons. The knockdown of calpain 1 and calpain 2 was achieved using adeno-associated viral vectors, AAV 2/1 serotype, expressing small hairpin RNAs targeting the individual calpain large subunits. The viral vectors were injected into the hippocampus 2 weeks prior to the ischemic insult. These data are in agreement with an in vitro study by Cao et al. (2007), who found that AAV-mediated knockdown of μ -calpain, but not m-calpain, protected primary rat cortical neurons from oxygen-glucose deprivation.

The in vivo findings of Bevers and colleagues nicely demonstrate the neuroprotective potential of the knockdown of individual calpain isoforms, although there are some caveats. The extent of knockdown was not quantified, thus the differences in efficacy of μ - versus m-calpain knockdown could possibly result from differences in remaining calpain levels. Their previous study demonstrated that knockdown of m-calpain protected primary rat hippocampal neurons against NMDA toxicity (Bevers et al., 2009). In the primary neuronal cultures, μ -calpain knockdown efficacy was difficult to evaluate due to low levels of μ -calpain protein and both m-calpain and μ -calpain shRNA constructs decreased μ -calpain mRNA levels to a similar extent (Bevers et al., 2009). The low level of μ -calpain in the primary hippocampal neurons is consistent with the developmental regulation of calpains (Li et al., 2009) and illustrates one challenge in directly translating in vitro findings to in vivo work in the adult CNS.

As discussed by Bevers and colleagues, the mechanisms by which μ -calpain knockdown contributes to neuroprotection remains to be determined. An increasing number of putative calpain substrates are implicated in the cell death resulting from calpain overactivation (Saatman et al., 2010; Bevers and Neumar, 2008). An additional issue is the potential for translating these exciting results to the clinic for treatment of neurologic disorders and acute CNS insults. While pre-treatment with isoform specific shRNAs delivered via viral vectors is unlikely to be a viable therapeutic strategy for acute CNS insults, delivery of shRNA or small interfering RNA (siRNA) is currently being investigated as a therapeutic for neurodegenerative disorders such as Huntington's disease (Mathupala, 2009; Pardridge, 2007; Pfister and Zamore, 2009). For calpains, an additional challenge is that the m- and μ -isoforms are long-lived proteins (Zhang et al., 1996), making it unlikely that knockdown of an individual calpain isoform following acute insults such as stroke or TBI would occur within a reasonable therapeutic window. The possible compensatory upregulation of other components of the calpain proteolytic system, or of other proteases, following isoform-specific knockdown will also need to be addressed in future studies.

In summary, knockdown of individual calpain isoforms using viral vector shRNAs is providing valuable information regarding both the pathological and physiological roles of individual calpain isoforms. The results of Bevers et al. (2010) indicate that knockdown of μ -calpain, one of several calpain family members in the CNS, may be sufficient to provide neuroprotection following acute insults such as stroke while sparing the essential physiological roles of other calpains. Isoform-specific calpain inhibitors are not yet

available, but represent a promising therapeutic strategy for stroke, traumatic brain and spinal cord injury, and a wide range of neurologic disorders.

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