Interaction Between Caspase-9 and Mutated Residues on the Third Domain (BIR3) of XIAP

Authors: T. M. Jeselun, S. I. Klosiewski, P. Lem, and J. L. Weis **Educators:** C. Ball, Ph.D. and H. Mernitz, Ph.D.
Institution: Alverno College
Mentor: Howard Jacob, PhD, Director, Human Medical Genetics Center, Medical College of Wisconsin

Abstract

The BIR3 (baculovirus IAP) domain of XIAP (X-linked inhibitor of apoptosis) plays a role in inhibiting the caspase cascade that initiates apoptosis. BIR3 has been shown to bind to caspase-9 to form a complex that inhibits apoptosis. Caspase-9, a homodimer made up of proteolytic and inactive monomers, is an initiator of the caspase mediated apoptosis. BIR3 prevents the dimerization of caspase-9. Four specific amino acids in BIR3 are important to apoptosis inhibition by the BIR3/caspase-9 complex. The structure and size of each conserved residue in BIR3 is predicted to affect the tight binding complex with caspase-9. A mutation in one of these amino acids, performed *in vitro,* does not stop the BIR3/caspase-9 complex formation, but the complex is no longer able to prevent caspase-9 homodimerization.

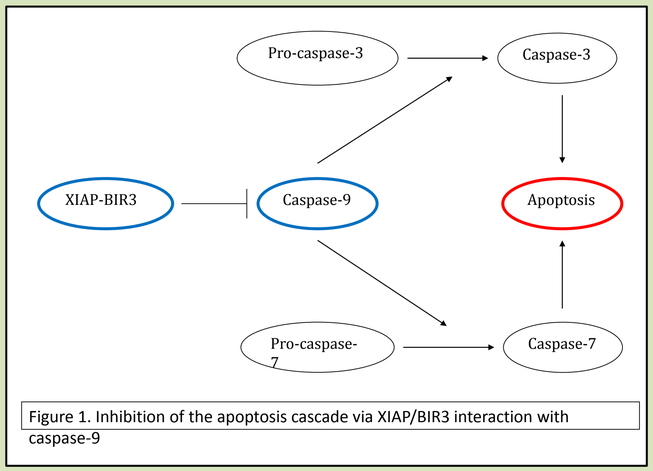

Figure 1. Inhibition of the apoptosis cascade via XIAP/BIR3 interaction with caspase-9

Introduction

XIAP is comprised of repeat baculovirus IAP domains (Marsh *et al*., 2010). The third baculovirus IAP (BIR3) regulates apoptosis by inhibiting caspase-9, an initiator protein which activates a cascade of events leading to apoptosis (Fig.1) (Renatus *et al*., 2001). Caspases are cysteine proteases that cleave substrates at an aspartate or glutamate residue. The focus of this study was on the complex formed by the interaction of BIR3 with caspase-9 (Fig.2) and how mutations in BIR3 can affect this interaction (Fig.3).

Mice with the caspase-9 gene knockout have low survival rates and severe brain abnormalities (Kuida, 1998), suggesting that caspase-9 is significant for normal function. A mutation in BIR3 that would increase the activity of caspase-9 would be likely to have significant results as well. Research into the topic did not present any *in vivo* BIR3 mutations that affected caspase-9; suggesting that either the mutation may not be viable or is insignificant. *In vitro* studies indicate specific BIR3 mutations greatly affect the protein's interaction with caspase-9 (Shiozaki *et al.,* 2003).

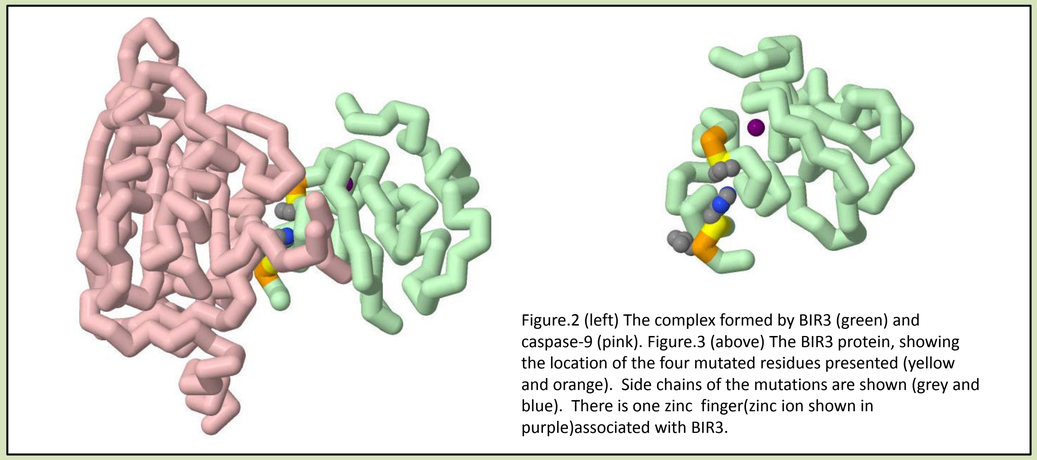

Figure.2 (left) The complex formed by BIR3 (green) and caspase-9 (pink). Figure.3 (above) The BIR3 protein, showing the location of the four mutated residues presented (yellow and orange). Side chains of the mutations are shown (grey and blue). There is one zinc finger(zinc ion shown in purple)associated with BIR3.

BIR3 and Caspase-9 Interaction

Our team's interest in XIAP in relation to caspase-9 was sparked by the story of Nicholas Volker, a young boy who suffered from irritable bowl syndrome and colitis due to a mutation within the XIAP gene. Understanding the interaction between caspase-9 and the BIR3 domain of XIAP will shed more light onto the pathway of apoptosis as a whole, including the mechanisms by which related illnesses occur.

Caspase-9 exists as a homodimer, with one active and one inactive subunit. The activity of effector caspases three and seven rely on caspase-9 for proteolytic processing (Fig.1). BIR3 attaches to the active caspase-9 when it has not yet formed a homodimer, blocking the caspase-9 active site and causing a change in caspase-9 conformation (Fig.4b) (Shiozaki *et al.,* 2003). When bound to caspase-9, the active loops of BIR3 are very similar to the loops of the inactive caspase-9 subunit. This allows for the tight complex of the BIR-3/caspase-9 heterodimer, inhibiting apoptosis (Shiozaki *et al.,* 2003). Hydrophobic alpha-helices are present on the surface of BIR3 and interact with the hydrophobic surface of caspase-9 (Fig.4a). The residues between the two proteins form 11 intermolecular hydrogen bonds and van der Waals forces (Shiozaki *et al.,* 2003).

The four point mutations P325G, G326E, H343A, and L344A interfere with BIR3 inhibiting apoptosis that is activated by caspase-9 (Fig.4a) (Shiozaki *et al.,* 2003). Another mutant is a multiple amino acid substitution in which glycine at position 326 is replaced with arginine (G326R), along with an H343Q and L344G exchange, have the same effect as the previous four mutations. This mutation converts a very small nonpolar residue to a much larger polar basic residue (Fig.5), interfering with the complex formation. These specific mutations destabilize the BIR3 interface and do not affect the groove on BIR3's surface, which allows caspase-9 to form a homodimer and, therefore, initiate apoptosis. While other inhibitors of apoptosis are also present, an increase in apoptosis would be expected if any one of the mutations were present (Shiozaki *et al.,* 2003).

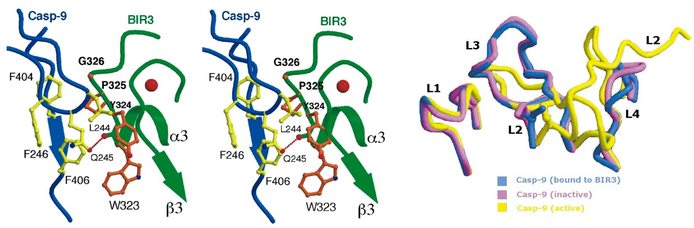

Figure 4a. (far left) Residue (yellow and orange) interactions between caspase-9 (blue) and XIAP-BIR3 (green). Figure 4b. Overlay showing change in caspase-9 conformation when bound to BIR3 (blue) vs. the active (yellow) and inactive (purple) dimer.
(Shiozaki *et al.*, 2003).

How Do We Know?

Shiozaki *et al.* explored how XIAP inhibits caspase-9 using protein crystallization techniques. They examined structures of the catalytic domains to predict how the BIR3 domain of XIAP recognizes caspase-9 and found that residues of the BIR3 domain pack tightly into one of the hydrophobic pockets of caspase-9. The crystal structures also suggested that van der Waals interactions work to secure the recognition of caspase-9 by XIAP-BIR3 and that the BIR3 domain holds caspase-9 in its monomeric form while also holding the active site loops in their inactive conformations (Shiozaki *et al.,* 2003).

The four residues of the BIR3 domain involved in the interaction of XIAP-BIR3 with the hydrophobic pockets of caspase-9 were further investigated in a mutational analysis with caspase-9 using its normal substrate, procaspase-3 zymogen (Shiozaki *et al.,* 2003). All four mutations of the BIR3 domain were shown to still heterodimerize with caspase-9 but without the ability to inhibit the caspase. This ultimately led to procaspase-3 being cleaved and activated in the apoptosis pathway.

What's the Next Question?

If one of the mutations (P325G, G326E, H343A, or L344A) discussed were to be tested *in vivo*, would the mutation be fatal? If not fatal:

- Would this increase apoptosis or would caspase-9 continue to be inhibited by other proteins?
- How would this mutation manifest itself pathologically?

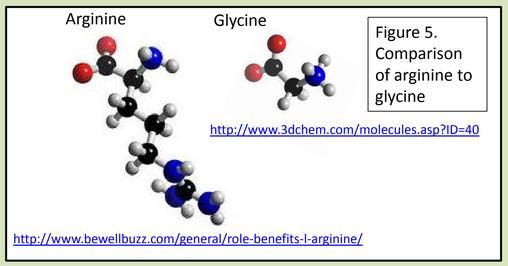

Figure 5. Comparison of arginine to glycine

Summary

The normal interaction of BIR3 and caspase-9 inhibits apoptosis. A mutation in specific residues can have a significant effect on this interaction, allowing for less regulated apoptosis to occur.

References

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