Cecilia Inés Zuliani
”Dr. sc.hum.”

The CD95/CD95L system and its role in the developing brain

Geboren am 12/07/1972 in Córdoba, Argentinien
Diplom der Fachrichtung Biochemie am 22/12/1994 an der Universidad Católica de Córdoba, Argentinien

Promotionsfach: DKFZ (Deutsches Krebsforschungszentrum)
Doktorvater: Prof. Dr. med. Peter H. Krammer

The CD95/CD95L system is primarily known for its function as a trigger of apoptosis. In the adult brain, this system is involved in the cell death following acute trauma or chronic neurodegeneration. In the developing brain, CD95 and CD95L are widely expressed and were thought to be involved in developmental apoptotic events. However, in mice lacking functional CD95 (*lpr*) or CD95L (*gld*), the number of cells was not increased in comparison *wt* mice. Moreover, overexpression of CD95L in 5-day-old mice did not increase the number of apoptotic cells. In addition, *in vitro*, CD95L was able to induce apoptosis only after 6-7 DIV and not at early stages. Thus, this system is not involved in the cell death found throughout the development of the brain.

In this study, we examined the role of CD95/CD95L in the developing brain. For this, we used hippocampal neurons in culture because of their well-defined polarization pattern. Treatment of these neurons with CD95L induced an increase in the number of branches. This increase in branching points was found both in the axonal and dendritic compartments. Also, endogenous CD95L increased branching in the hippocampal neurons. This phenomenon could be inhibited with an antibody neutralizing CD95L. In addition, time-lapse studies revealed that the branching increase was due to the formation of new branches rather than inhibition of the retraction of previously formed branches.

CD95L transduces the apoptotic signal through binding to its receptor. CD95 requires its death domain (DD) to trigger apoptosis. In young neurons from mice lacking a functional CD95 or with a mutated death domain (*lpr-cg*), CD95L was not able to induce increased branching. Thus, CD95L signals branching through CD95 and its death domain.

Caspase-8 is recruited to CD95 via its DD, and subsequent activation of effector caspases takes place. In our system, even if CD95 signals branching through its death domain, caspase activation was not involved. Interestingly, CD95L treatment did not increase caspase-3 cleavage.

The best-studied molecules involved in the regulation of neuronal shape are the members of the Ras superfamily of small GTPases. Triggering of CD95 in young neurons increased the level of activated Ras. Ras activation leads to phosphorylation of the Akt-GSK3β pathway. GSK3β phosphorylation (Ser-9) and consequent inhibition upon CD95L induces branching increase. Overexpression of a form of GSK3β, in which phosphorylation of Ser-9 is not possible abolished the capacity of CD95L to induce branching.

Finally, we investigated whether the lack of CD95 affected branching *in vivo*. Interestingly, the lack of CD95 signaling results in reduced branching of the apical and basal dendritic trees. Similarly, cerebellar Purkinje cells from *lpr* and *gld* mice had less and thinner branches than *wt* mice.

Understanding how CD95L signals for the different phenotypes would provide a potent tool for the treatment of acute brain trauma and neurodegenerative diseases.