Apoptosis is necessary for maintaing the integrity of all alive multicellular organisms and therefore needs to be precisely regulated. Very important regulators of apoptosis are pleiotropic cytokines from TFGβ superfamily (e.g. TGFβ, BMP, aktivins), whose signals are transduced by SMAD proteins.

Patients with secondary myelodysplasias and acute myeloid leukemias (MDS/AML) frequently exhibit interstitial deletions of the chromosome-5q resulting in hemizygous loss of the transcription transactivator SMAD5. SMAD5 is a member of the signal transducer family conveying the pleiotropic TGFβ/BMP cytokine signals with roles in development, cell growth control, and tumor progression. Consistent Smad5 gene expression in these cell types and the gradual increase in its mRNA and protein levels in a model of induced erythroid differentiation of murine erythroleukemia (MEL) cells suggest a role of the gene in hematopoiesis. We show that bone morphogenetic protein 4 (BMP4) directs Smad5 activation in human hematopoietic cells, as monitored at the levels of protein phosphorylation, nuclear translocation, and specific transcription response. In vitro induction of normal human CD34+ cells by BMP4 results in significantly increased proliferation of erythroid progenitors (BFU-E) and formation of glycophorin- A+ cells, whereas perturbation of Smad5 expression by antisense oligonucleotides causes significantly decreased rates of BMP4-induced erythroid differentiation. We have not detected any effects of Smad5 inhibition on BMP4-stimulated progenitors of the granulocyte–macrophage lineage. We propose that the BMP4/Smad5 signal transduction pathway activates hematopoietic differentiation programs that may be impaired in anemia manifestations in MDS and AML patients with Smad5 haploinsufficiency.

Next to regulatory proteins as TGFβ/BMP cytokines and their signaling transducers inducing (but in some cases also inhibiting) apoptosis, cell differentiation and proliferation, there are proteins with distinct antiapoptotic role as Hsps. But even these strong cytoprotective agents might get in certain situations exhausted (when the cell is beyond recovery damaged and so potentially dangerous for the whole body) and can destroy the cell by apoptotic mechanism for saving the whole organism.

Heat shock proteins (Hsps) are involved in multiple cellular processes during normal and stress conditions, particularly in the folding of polypeptides. A newly recognized property of the members of the Hsp70 family is their ability to interact with lipids, opening ion conductance pathways in artificial membranes, and integrating into natural membranes. The formation of Hsp70 channels in biological membranes and their function is still elusive. We show that Hsp70 and Hsc70 display a highly selective interaction with phosphatidylserine moieties on membranes, followed by rapid incorporation into the lipid bilayer. Addition of Hsp70 or Hsc70 into the extracellular medium resulted in a viability decrease of cells beading PS on the exterior surface, such as PC12 cells. This toxic effect is modulated by the presence of ATP or ADP and can be blocked by screening PS moieties with AnnexinV. These observations suggest that the presence of Hsp70 in the extracellular medium may be an accelerator of apoptosis since the presence of PS on the surface is an early indicator of this process. These findings may also explain the toxicity observed in cells overexpressing Hsp70s and provide a rational for the tight regulation of Hsp70 expression.

Apoptosis occurring in the wrong place and/or the wrong time or not at all can lead to serious diseases. Apoptosis of neurons associated with accumulation of Aβ is observed in Alzheimer's disease whose mechanism remains still unknown. Dr. Arispe's hypothesis of AD describes apoptosis of neuronal cells as a consequence of calcium influx mediated by Ca channels formed by Aβ inserted in the plasma membrane.

Extracellular application of the Alzheimer's Aβ peptide evokes a series of cellular responses that leads to the death of cells by apoptosis. While it has been shown that some responses to freshly prepared Aβ occur immediately, such as changes in intracellular calcium concentration and changes in membrane permeability and phosphatidylserine asymmetry, we show for the first time that the cytotoxic action of externally applied Aβ such as caspase activation and apoptotic loss of cell viability, persists even several days after Aβ is removed from the medium. We also show that the mechanism for this persisting cytotoxic action of externally applied Aβ is based on the sustained activity of active Aβ ion channels that remain incorporated into the cell membrane. To confirm this assessment the classically known Aβ channel blockers zinc and tromethamine, and a new customdeveloped short peptide segment from the sequence forming the mouth of the Aβ □channel that very effectively blocks Aβ Ca2+ channels were used. This is the first report of a specific Aβ channel blocker compound, NA4, which efficaciously and potently blocks the most known cellular responses to Aβ.

The major component of amyloid plaques in Alzheimer's diseased (AD) brains, the Aβ disrupts intraneuronal homeostasis and generates neurotrophic and neurotoxic effects. The internal ten residue fragment Aβ25-35 has been reported to be the active fragment of Aβ □because it exhibits similar neurotoxicity. Consequently, Aβ25-35 has been widely used experimentally as a model peptide to investigate the cytotoxic properties of Aβ. However, numerous reports have also stressed functional differences between Aβ and Aβ25-35. To test this Aβ/Aβ25-35 equivalence hypothesis, we have used in vitro and in vivo assays to simultaneously test both peptides for membrane interaction and activation of pre-apoptotic events. Our data on membrane integrity, caspase activation and DNA fragmentation show that there are many fundamental functional differences between Aβ25-35 and Aβ40. We conclude that Aβ25-35 and Aβ40 have quite distinct cytotoxic mechanisms. These data support the concept that the internal Aβ25-35 peptide fails to model the properties of the natural Alzheimer's Aβ.