Biochemical and Biophysical Research Communications 482 (2017) 445–449

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Essay

Cell death: From initial concepts to pathways to clinical applications – Personal reflections of a clinical researcher

Klaus-Michael Debatin

Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Eythstr. 24, 89075 Ulm, Germany

ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form
24 October 2016
Accepted 27 October 2016

Keywords:
Apoptosis
Cell Death
IAPs
CD95 (APO-1)

ABSTRACT

Cell death research has come a long way from the description of cellular phenomena to identification of pathways to the role of apoptosis in tissue homeostasis in the pathophysiology of diseases and the delineation of targets for therapeutic intervention. In this very personal view of a clinical researcher in the field from the very beginning to recent clinical application, essential elements of cell death research over the past decades are seen from a perspective of development of science and medicine.

It was around the mid 80s, having vacation with the family and sitting on a mediterranean beach doing what researchers do in holidays: reading papers. I was excited by a publication of Thomas Waldmann's group at the NIH [1,2], describing an antibody produced for identification of a leukemia specific marker that (by chance) appeared to recognize the IL-2 receptor, a cytokine recently discovered and implicated in the growth of normal and malignant T cells. Binding of the anti-Tac antibody seemed to inhibit proliferation of these malignant T cells. I was not only struck by the fact that proliferation could be inhibited by an antibody to a cell surface receptor, but also by the fact that his group, using malignant cells to generate monoclonal antibodies against putative leukemia-specific cell surface molecules, by chance found an antibody that interfered with growth of malignant cells. A typical project at this time, primarily designated to characterize cell surface molecules on malignant cells, contributed to a concept that malignant cells might require growth factors for proliferation.

Research in hematology/oncology in the 80s was dominated by the search for oncogenes that drive proliferation as the causative event of malignant transformation [3,4]. Cell proliferation was considered as a tightly regulated process involving the machinery for cells to divide, as well as the response to external signals that instruct cells to enter the cell cycle and grow. Thus, a number of cytokines and their receptors, in particular in lymphohematopoiesis, were described. As a young clinical researcher I was attracted to the concept of cytokine mediated growth of leukemia cells. I realized, however, that ambitious projects can only be performed in the context of an appropriate scientific environment and, therefore, started to work at the German Cancer Research Center, as one of the few people dealing at that time with human cells, where I entered Peter Krammer's laboratory, a cytokine place in those days.

I was busy with understanding the regulation of proliferation in primary leukemia cells from my patients. I remember very well the night, when one of our patients died, due to progressive leukemia. I was on night-shift and returned to the lab, where I was trying to culture the malignant cells of this 6-year-old boy and I still remember the patient and my immediate dedication to understand, what keeps these cells growing. Having leukemic cells in my laboratory that at least on short-term culture responded to enigmatic growth factors present in supernatants produced by e.g. activated T cells, I thought that the “Waldmann-approach” could also be used to detect cytokine-mediated growth of leukemic cells. In addition to paracrine signaling, where the tumor/leukemia cell is responding to growth factors produced by their neighbours, a concept of autocrine growth emerged, in which leukemic cells are themselves the producers of growth-promoting cytokines to which they respond. This idea was the basis for the start of experiments that we initiated to generate monoclonal antibodies against leukemia cells with the idea to inhibit proliferation. That cells die was at this time considered to be just a consequence of lack of

E-mail address: klaus-michael.debatin@uniklinik-ulm.de.

http://dx.doi.org/10.1016/j.bbrc.2016.10.124
0006-291X/

something, an accident of cell culture or a process *in vivo* that was produced by harsh intervention using cytotoxic drugs or other forms of cellular cytotoxicity occurring as an accident, such as lack in oxygen supply, nutrition supply etc. No one thought about cell death as a possibly regulated cellular feature similar to cell growth and differentiation. Generating myriads of hybridomas against leukemia cells did not generate an antibody that fulfilled the job in inhibition of proliferation in the first place. However, by similar approaches, although for a different purpose, we eventually came down with an antibody that inhibited proliferation of the target B-lymphoblastic cell line [5].

It soon turned out that this antibody not only blocked cell growth, but also induced a unique cellular phenotype associated with cell death. Apoptosis at that time was not really "on the scene", but we soon realized that features of apoptosis were induced by this antibody now called "anti-APO-1". An initial idea that I had was that this antibody might block ion channels and, therefore, I contacted researchers dealing with ion channels to ask how cells with blocked ion channels would look like. It soon turned out that a similar antibody was produced by another group in Japan [6]. One of the features, induced by anti-APO-1 in the leukemic cells, was associated with a hallmark of this particular form of cell death called "apoptosis", the fragmentation of DNA in form of a DNA ladder [7,8]. It also turned out that the APO-1 receptor was indeed a death receptor mediating apoptosis. I spent months to analyse different cells treated with the anti-APO1 antibody to count dead cells and to isolate DNA for showing DNA laddering, the cutting of DNA at specific sites at the histones to produce characteristic multimers of DNA fragments. Due to the interest of the Waldmann laboratory and a general interest in antibodies that block proliferation, I went to the NIH, studied the very same cells with which Tom Waldmann's group has shown expression of high levels of the IL-2 receptor alpha-chain and the inhibition of proliferation by the anti-Tac antibody to analyse these cells for sensitivity to our new antibody. This led to the first description of apoptosis induction in human malignant cells [9,10].

The late 80s, early 90s were in retrospect an absolutely exciting area of research, where completely different fields contributed to understanding what cell death could be. These diverse fields included the discovery of a gene involved in the t14; 18 chromosomal translocation in follicular lymphoma, an approach abundantly used to characterize oncogenes that led to the discovery of the Bcl-2 proto oncogene [11]. Bcl-2 soon turned out not to be primarily a driver of proliferation, but rather an inhibitor of the natural process of apoptosis, inbuilt in all cells of the body, in particular in cells of the hemato-lymphopoietic system [12–15]. It also included completely different work performed in developmental biology in c.elegans that led to the discovery of anti-death and pro-death genes [16–18]. Then the enzymatic machinery called caspases that execute the apoptotic cell death program was identified [19–22]. These discoveries were preceded by the first description of apoptosis as a basic cell phenomenon years before pathways were described [8,23]. Meetings of the 90s brought together researchers from many different areas, and I had the privilege as a clinical researcher to contribute to the field and attend meetings with researchers clinicians usually do not have a contact with. In addition to the core apoptosis machinery with its prototype protagonists, identified during the 90s, from the very beginning a number of related or associated cellular events were in the focus of researchers in the cell death area. This included cellular changes associated with transglutaminase expression and activity [24], the analysis of the mechanisms underlying cancer therapy-induced cell death [25–27], the role of mitochondria [28–30] and the contributions that identified p53, not only as a hallmark mutation in cancer, but also its direct and indirect relationship to cell death programs such as apoptosis [31,32].

When I first met Sten Orrenius. I did not know much about toxicology, other than that toxicology researchers dealt with substances and cellular events associated with some sort of disturbance of cell function and cell killing. It was in September 1993, when a group of researchers met at Château Maffliers near Paris at one of the first cell death conferences, which turned out to be the 1st Euroconference on apoptosis, to exchange the scientific results and discuss concepts, how the field could move further. It was mostly a European meeting along with US participants and also major contributions from Australia. To drive the field further, it was decided to start with Euroconferences on apoptosis. The major contribution of Sten Orrenius to the development of the field, that I realized at that time, was on two different levels. First of all, as a toxicologist he opened my eye for intracellular processes, such as calcium fluxes and Redox signaling associated with deregulation of the cellular homeostasis and cell death mechanisms [33–35]. His second contribution was due to his standing as a professor of Karolinska Institute as a world-renowned scientist, not only in the toxicology field, and as a person of great integrity who never worked with the perspective of his own profit. With this "backbone" Sten managed to get the European cell death meetings and eventually the European Cell Death Organization (ECDO) going. I also enjoyed personal support by Sten at various levels, which I only realized later, that brought me into contact with institutions worldwide that sought advice and help, how cell death research and molecular oncology in particular could be brought forward in medicine at a time, where "translational medicine" was not even mentioned in discussions.

In deciding, whether to follow a pure researcher or a clinical researcher path, I decided to dedicate my own work to follow up on the early stimulation that I got from my patients in trying to understand the mechanism, by which malignant cells, and in particular leukemia cells, could be killed. The late 80s and the early 90s also marked the success in anticancer therapy and in anti-leukemia therapy in particular, especially in children. Due to multidrug chemotherapy and a highly organized setting, the cure rates in children and in particular with acute lymphoblastic had steadily increased and reached a stable plateau in the 90s. Since then it was a general agreement that further intensification of chemotherapy would not add more to the cure rate of almost three-fourths of affected children. In studying the action of cytotoxic drugs on leukemia cells, a classical toxicology area in the context of apoptosis signaling, we soon realized that the molecules that are involved in cell death pathways may also play a key role in determining sensitivity and resistance of leukemia cells to cytotoxic drugs, and apoptosis pathways may even be triggered by drugs formerly considered to simply "kill" cells [27,36,37]. Based on the discovery of the role of the CD95/APO-1/Fas system in the normal homeostasis of T cells and the discovery of Fas mutations in lpr mice, we also realized that mutations in the CD95/APO-1/Fas gene may lead to a disease, called auto-immune lympho-proliferative syndrome that resembled the lpr/gld phenotype in mice [38–41]. The elimination of normal T cells during immune responses by activation of the APO-1 system, called autocrine suicide, where T cells produce the ligand for CD95 and at the same express the receptor, thereby killing themselves, was also instrumental in trying to understand at least some features of HIV infection [42–44]. Indeed, CD4 T cells in HIV exhibit an activated APO-1 system and interfering with binding of the CD95 ligand to its receptor, was able to block apoptosis *ex vivo* in T cells from HIV-infected individuals.

With the unique feature of inducing apoptosis in cancer cells, there was of course a great hope that the CD95 antibody, anti-APO-1 or its natural ligand would represent a novel approach to tumor therapy. In search for a model to analyse the CD95 system, I have