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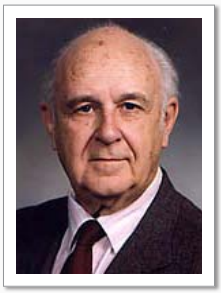

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From the first successful gene transfer into human cells to the present status of gene therapy – Celebrating the 50th Anniversary

Introduction: Inspired by genetic experiments with prokaryots of the 1940-50 decades, as exemplified by presentations at the CSHL Bacteriophage Meetings and analogous gatherings, we decided about half century ago to develop an analogous field of human cell genetics by first, isolating well defined and easily selectable mutants of human cells, and then, designing and performing experiments to study mutations, mutagenicity, recombinations and DNA-mediated transformations or transfections. Concerning the latter, our aim was to determine whether we could apply the DNA-mediated gene transfer to human and animal cells, as was originally shown in 1944 for bacteria by Avery, MacLeod, and McCarty (Journal of Experimental Medicine 79 : 137–158)

HAT: Using gene HPRT that encodes the enzyme hypoxanthinephosphoribosyl transferase, (HPRT), we developed a system permitting selection of forward and reverse mutants. We have shown that selection for the 8-azahypoxanthine(azaH)-resistance selects for forward mutants that have lost their HPRT enzyme and thus can neither use nor be inhibited by azaH. Reciprocally, the reverse mutants or transformants that have acquired the HPRT gene were selected as the only being able to form colonies on our HAT medium (hypoxanthine + aminopterin + thymidine), in which the purine biosynthesis pathway was blocked by aminopterine.

The first gene transfer: In 1962, using this system, we have isolated several azaH-resistant mutants of human Detroit-98 bone marrow cell line, which have lost the HPRT gene activity and were not able to form colonies on the HAT medium (Szybalska E and Szybalski W. Proc. Natl. Acad. Sci. 48, 2026-2034). These cells, and especially the D98/AH-2 line, which has never produced any revertants that grew on the HAT medium, were exposed to DNA purified either by the phenol method or by the CsCl density-gradient centrifugation. When Ca.phosphate-precipitated DNA was used, colonies were formed over nearly 1000-fold DNA concentration range. DNA isolated from transformants had similar transducing activity as that extracted from the wild-type Detroit-98 cells. Transducing activity was insensitive to RNases but sensitive to DNases.

Gene therapy *in vitro*: About two years after our discoveries, a neurological "syndrome", based on the loss of the HPRT enzyme was discovered in children by Lesch and Nyhan,(Am. J. Med. 36, 1964, 561-570). It is obvious that our azaH-resistant D98/AH cells generated in vitro were acquiring the Lesch-Nyhan-like "syndrome" (even before this syndrome was ever identified in children). Furthermore, we realized that we were able to "cure this syndrome in vitro" by transfection with the Ca.phosphate-precipitated HPRT+ DNA employing HAT selection. Thus, we described the first model of the human gene therapy, based on our successful and well documented DNA-mediated transformation/transfection of the eukaryotic cells.

Hybridomas and monoclonal antibodies: HAT selection permitted me also to construct the first ever hybridoma cells by crossing our HPRT-defective D98/AH line with cells from the fresh explant of my own HPRT+ skin cells and also later development of monoclonal antibodies.

Present status: At present, gene therapy, defined as a techniques for correcting defective genes responsible for diseases, is a mainstay for a multitude of laboratory experiments with various organisms and tissues. However, it is still in its infancy regarding human clinical research and its applications, because of a plethora of various ethical and technical considerations. The Lesch-Nyhan-like "syndrome", although "created and cured" in human cells nearly half century ago (Szybalska and Szybalski, 1962), was never yet successfully treated by gene therapy in clinical settings. We know now, that the absence of HPRT in the early developmental stages leads to irreversible neurological changes. Moreover, the Food and Drug Administration (FDA) has never approved any human gene therapy product for sale.

Gene transfection is presently a routine in cell cultures and animals. Therefore, it is my current conviction that the large scale clinical-like gene therapy research should be carried on animals. For human in clinical settings one could treat and transfect patient's genes, but only when carried in explanted cells. Only after such patient's cells with treated genes are exhaustively tested, including their DNA sequencing and identification, the re-implantation into the patient of such products of gene therapy might be considered.

Biography

Waclaw Szybalski is the Father of Gene Therapy. With his team, they were the first ever, who transformed the human cells with the externally engineered transgenes (Szybalska, E.H., Szybalski, W., 1962. Genetics of human cell lines. IV. DNA mediated heritable transformation of a biochemical trait. Proc. Natl. Acad. Sci. 48, 2026-2034). This work created the foundations for what is now called gene therapy. He the author of 232 peer-reviewed articles on the subject. He earned his Doctoral Degree at the University of Gdansk. Poland, EU. He is currently a professor of Oncology at the McArdle Laboratory for Cancer.

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