

Tissue culture: the unrealized potential

Gordon Sato

Received: 10 September 2007 / Accepted: 22 October 2007 / Published online: 11 December 2007
© The Author(s) 2007

Abstract Lack of differentiated functions of the tissue of origin in tissue culture thought to be due to dedifferentiation was shown to be due to selective overgrowth of fibroblasts. Enrichment culture techniques, (alternate animal and culture passage), designed to give the functionally differentiated cells selective advantage over the fibroblasts resulted in a large number of functionally differentiated clonal strains. Thus the dogma of dedifferentiation was destroyed. It is proposed to substitute the dedifferentiation hypothesis with the hypothesis that cells in culture accurately represent cells in vivo without the complex in vivo environment. With the development of hormonally defined media, combined with functionally differentiated clonal cell lines, the potential of tissue culture studies is greatly augmented. Hormonal responses and dependencies can be discovered in culture and the discovery of dependencies of cancer cells has led to a new rationale for therapy.

Keywords Alternate animal and culture passage · Enrichment culture · Dedifferentiation, hormonally defined media · Selective overgrowth

It is an honor and great privilege to deliver the Murakami Memorial lecture. Hiroki Murakami spent 2 years in my laboratory where he learned of the great potential of tissue culture, and was inspired to form a Japanese Society that could exploit its many possibilities. I helped Hiroki overcome the opposition of existing societies by bringing distinguished American lecturers to the first JAACT meeting. The subject of papers presented at this meeting represent the important role of cell technology in solving human health problems. Hiroki would be pleased at the evolution of JAACT and the impressive prospects for its future. I believe JAACT will continue to be a great force in the development of cell science in Japan.

My objective in 1957 when I first entered the field of tissue culture was to study the basic element of the animal, the cell, in isolated, controlled conditions, tissue culture, to learn the details of animal physiology. The trouble with this simple idea in 1957 was that animal cell cultures rarely exhibited the differentiated properties of the tissue of origin. It was universally believed that cells in culture underwent dedifferentiation to become a common cell culture type. In other words, cells in culture were different from cells in the animal. It was crucial for tissue culture to determine if this was true. In 1960, we did the only experiment ever performed to determine if the lack of differentiated properties in culture was due to dedifferentiation or selection of a minority cell type in the inoculum, the connective tissue fibroblast. In this experiment freshly isolated mouse liver tissue

G. Sato (✉)
A and G Pharmaceutical Corp., 27 Cedar St., Wenham,
MA 01984, USA
e-mail: manzanarmangrove@hotmail.com
URL: www.manzanarprojectfoundation.com

was divided into three portions. The first portion was untreated, the second portion was treated with specific anti-liver parenchyma antisera and complement, and the third portion was treated with specific anti-fibroblast antisera and complement. Treatment with anti-liver parenchyma antisera and complement resulted in a great amount of complement fixation. After brief treatment the antisera and complement were washed away and the inoculum plated in culture. No effect of this treatment was noted, as the subsequent growth was equivalent to the untreated control. When the inoculum was treated with anti-fibroblast antiserum, complement fixation was negligible as very few fibroblast were present in the inoculum, but subsequent growth was completely inhibited. The lack of liver properties in culture was not due to dedifferentiation but to selective over growth of fibroblasts. This should have settled the matter, but publication of this paper (Sato et al. 1960) brought me a great deal of abuse. Members of the tissue culture association rhetorically announced that they would buy me a microscope so I could see liver cells turn into fibroblasts, and the American Cancer Society in response to my application for a grant wrote me that I should never again apply to the society for a grant. Often scientists find it difficult to accept the demise of a long standing notion which they have come to accept as fact even when presented with data demonstrating that this “fact” cannot be true. Now 45 years after my controversial publication young scientists are unaware that dedifferentiation was once dogma.

The next step was to develop enrichment culture techniques which would give the differentiated cells some selective advantage over fibroblasts. This was accomplished by using functionally differentiated animal tumors and alternately passaging these tumors through culture and animal. The rationale was that each passage through culture would select culture hardy variants of the functionally differentiated tumor cells and these would be able to compete with fibroblasts in culture. In this way we established steroid secreting and ACTH responsive adrenal tumor cells and ACTH secreting pituitary tumor cells (Buonassisi et al. 1962). By this method, within 10 years we had established functional clonal lines of steroid secreting adrenal cells, ACTH secreting pituitary cells, growth hormone secreting pituitary cells, antigen specific glial cells, neuroeffector

synthesizing neuroblastoma cells, differentiating teratoma cells, pigmented melanoma cells, androgen secreting testicular cells etc. Thus the dedifferentiation hypothesis was destroyed. Fresney in his book on cell culture says the dedifferentiation hypothesis died by itself. I believe his thinking is limited like the early tissue culture establishment that thought I should get a microscope so I could see liver cells change into fibroblasts. Not only does dedifferentiation not occur, but I would also like to put forward the hypothesis that cells in culture accurately represent cells in the animal body, and that discoveries made on cells in culture are directly related to animal physiology.

At this point it was pointed out to me by Kiyoshi Ueda, a post-doctoral fellow in the lab, that no cell cultures existed whose proliferation was driven by hormones, as were cells in the animal. We set out to develop cells in culture whose proliferation would be driven by hormones by following the procedure of Biskind and Biskind (1944). They implanted rat ovary tissue in the spleen of ovariectomized rats. The spleen is drained by the hepatic portal vein and any steroids secreted by the implant are destroyed by the liver. The pituitary sensing a deficiency of steroids secretes gonadotrophins causing the ovarian implant to grow. We established such ovarian cells in culture and their growth was dependent on the addition of some crude NIH Luteinizing Hormone. At this point the human factor began to complicate things. We got pure LH from Dennis Gospodarawicz and it was inactive. He wanted to purify the new factor using our ovarian cells, but I had a Japanese, protein chemist, post-doctoral fellow coming and I wanted him to purify the factor. Another post-doctoral fellow in my laboratory found that the crude LH stimulated 3T3 cells to grow and he wanted credit for discovering the new factor, so secretly he was meeting with Dennis to work with him. Meanwhile my grant was turned down on the grounds that pure luteinizing hormone was not active and a subsequent grant that showed that hormones could replace serum was turned down on the grounds that just because one cell could have its serum replaced by hormones did not mean that this would be true for all cells. My interpretation was that the person who turned down my grants had previously worked in my lab and wanted to be known as the person who brought cell culture to endocrinology. It was simply a matter of poisonous competition, and

Crude LH which was the source of FGF and hormonal replacement of serum would not be funded. Thus my lab which had made important innovative discoveries was in financial trouble.

About this time, I went on sabbatical leave to the Basel Institute of Immunology because I had been an admirer of Niels Jerne from my graduate school days at Cal Tech. During my stay in Basel, I was asked to write an essay on what I thought the function of serum was in tissue culture (Sato 1975). In this essay, I proposed that the function of serum was to provide hormones. A year later, Izumi Hayashi in my lab showed this was true for GH3 cells that produced growth hormone (Hayashi and Sato 1976). This principle has proven to be generally valid and represents a key advance in endocrinology, and yet the Endocrine society has not seen fit to devote a session to this subject. The culture approach uncovers many more responses by cell types to hormones and uncovers hitherto unknown hormones (growth factors). The responses found in culture should be tested in the animal and the so-called growth factors should be tested *in vivo*. This approach should reveal hitherto unknown complexities, which are necessary to explain necessary subtle control mechanisms. Autocrine hormones are discovered by the culture approach. The Cancer society has not devoted a session to hormonally defined media although it will be central to developing therapies in the future. The cultures of liver parenchyma and embryonic stem cells have been difficult in the past (Martin 1981; Ichihara et al. 1980). With the development of hormonally defined media, fibroblastic overgrowth is eliminated and the media could be designed to support only the growth of the desired cell. Establishment of cell types previously considered difficult should become routine.

The discovery of hormonally derived media caused me to think about hormonal requirements of cancer cells and possible approaches to therapy (Sato 1980; 1981). In these articles, I mused about hormonal dependencies of cancer discovered in culture and proposed possible therapies by blocking the hormones by antibodies to their receptors. Normal tissues deprived of their trophic hormones shrink, but after the hormone is restored the normal tissue regains its original size. I believe that a cancer cannot survive deprivation of its hormone. I draw this conclusion from analogy with the experiment of Françoise Kelly

(Kelly and Sambrook 1973). She treated 3T3 and SV transformed 3T3 (SV3T3) with cytochalasin B, which blocks cell division. Normal 3T3 when treated with cytochalasin makes a binucleate cell and then stops nuclear division. When cytochalasin is removed the binucleate divides to form two mononucleate cells and then divides normally. When SV3T3 is treated with cytochalasin, the cell cannot divide but the nuclei continue to divide to form a multinucleate cell, and when the cytochalasin is removed the multinucleate is non-viable. I believe that this is a general principle. When one process is inhibited the normal cell can coordinately inhibit other processes that if allowed to continue would result in a non-viable situation. The cancer cell cannot do this. Therefore, I believed if we could inhibit one hormone dependent process in a cancer cell that this would result in the death of the cancer cell. In time, I believe that this will become a general principle concerning the vulnerability of cancer cells. I thought to make anti-EGF-receptor antibody because I knew of long acting thyroid factor which was an antibody to the TSH receptor so that antibodies to hormone receptors were possible, and the EGF receptor had been purified by Stanley Cohen (Cohen et al. 1980). The work was done by my son, Denry Sato, and a post-doctoral fellow, Tomoyuki Kawamoto. The concept of hormone therapy was well formulated and the plan of work was well thought out when John Mendelsohn became a self-invited guest in our laboratory observing the work with interest. The antibody could block growth in culture of A431 cells in culture and block the formation of tumors in nude mice. This is confirmation that cells in culture accurately represent cells *in vivo*. At this point, I was busy taking over the direction of an Institute in Lake Placid NY. Unknown to us John Mendelsohn got a company to commercialize the product and develop a humanized version of the antibody for human therapy. This has proven to be successful and now is the biggest money earner for the University of California at San Diego. The development of a monoclonal antibody therapy to cancer depends on a long series of experiments. First of all dedifferentiation did not occur. Clonal lines of functionally differentiated cells had to be grown which showed that cells in culture accurately represented cells in the animal. The hormonal requirements of cells (tumor) had to be determined. Monoclonal antibodies to a hormone receptor had to be produced. These

antibodies killed cells in culture. The next step was to show that the tumors in nude mice were also killed. Fortunately, I had established a nude mouse colony with great effort in my laboratory (Masui et al. 1981). The general validity of this approach is demonstrated by the remarkable work of Ginette Serrero on breast cancer, which began in the W. Alton Jones Cell Center and continues at A. and G. Pharmaceutical Corp. (Serrero 2003). In culture, it was discovered that pituitary cells that secrete growth hormone respond to TRH by secreting prolactin. In animals it was shown that TRH causes the secretion of prolactin (Tashjian Jr 1979). In the future, I expect many more hormonal responses to be discovered in tissue culture.

At Lake Placid, I tried to form an Institute, the W. Alton Jones Cell Science Center, that develops and supports young scientist. The model, I had in mind was the old Kaiser Wilhelm Institutes. The scientists were freed from the poisonous need to compete for credit and financial support. They worked for the love of science and the understanding that one of the moral objectives was to improve the human condition. To this end, Wally McKeehan and I formed Upstate Biotechnology to support the institute. This company was recently sold for 200 million dollars. I almost succeeded. The Jones Foundation closed the scientific center and kept the company. However, my vision has been instilled in many of my students, and someday they will form the ideal, self supporting institute where scientists work for the love of science and where advances in biology will be made that better the human condition.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Biskind MS, Biskind GR (1944) Growth of ovarian implants in spleens of ovariectomized mice. *Proc Soc Exp Biol Med* 55:176

- Buonassisi VG, Sato G, Cohen AI (1962) Hormone producing cultures of adrenal and pituitary tumor origin. *Proc Nat Acad Sci USA* 48:1184–1190
- Cohen S, Carpenter G, King L Jr (1980) Epidermal growth factor receptor-protein kinase interactions. Co-purification of receptor and epidermal growth factor-enhanced phosphorylation activity. *J Biol Chem* 255:4834–4842
- Hayashi I, Sato G (1976) Replacement of serum by hormones permits the growth in a defined medium. *Nature* 259:132–134
- Ichihara A, Nakamura T, Tanaka E, Tomita Y, Aoyama R, Sato S, Shinno H (1980) Biochemical functions of adult rat hepatocytes in primary culture. *Ann NY Acad Sci* 349:77–84
- Kelly F, Sambrook J (1973) Differential effect of cytochalsin B on normal and transformed mouse cells. *Nature New Biol* 242:217–219
- Martin GR (1981) Isolation of a pluripotential cell line from early mouse embryos cultivated in a medium conditioned by teratoma stem cells. *Proc Nat Acad Sci USA* 78:7534–7638
- Masui H, Kawamoto T, Sato JD, Wolf B, Sato G, Mendelsohn J (1981) Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res* 44:1002–1007
- Sato G (1975) The role of serum in cell culture. In: Litwak G (ed) *Biochemical Action of Hormones, VIII*. Academic Press, Inc, New York, San Francisco, London, pp 391–396
- Sato GH (1980) Iacobelli S et al. (eds) *Towards an endocrine physiology of human in cancer hormones and cancer*. Raven Press, New York
- Sato GH (1981) Antibodies, hormones, and cancer. In: Takacodi Lorenzo C (ed) *The immune system. Vol I*. S. Karger, Basel Switzerland, pp 379–382
- Sato G, Zaroff L, Mills SE (1960) Tissue culture populations and their relation to the tissue of origin. *Proc Nat Acad Sci USA* 46:963–972
- Serrero G (2003) Autocrine growth factor revisited, PC cell derived growth factor is critical in breast cancer tumorigenesis. *Biochem Biophys Res Commun* 308:409–413
- Tashjian AH Jr (1979) Clonal strains of hormone producing pituitary cells. *Methods enzymol* 58:527–535