CC/NUMBER 49
DECEMBER 3, 1990

This Week's Citation Classic®

Svoboda J, Chyle P, Šimkovič D & Hilgert I. Demonstration of the absence of infectious Rous virus in rat tumour XC, whose structurally intact cells produce Rous sarcoma when transferred to chicks. *Folia Biol. Prague* 9:77-81, 1963.
[Inst. Experimental Biology and Genetics, Czechoslovak Acad. Sciences, Prague, and Inst. Oncology, Bratislava, Czechoslovakia]

The permanent presence of noninfectious but rescuable Rous sarcoma virus (RSV) genome in all *in vivo* or *in vitro* passaged XC cell lines or clones, in the absence of any signs of infectious virus formation, led to the conclusion that XC cells are virogenic and harbor RSV provirus. It was proposed and later proven that the virus rescue from XC cells was based on fusion of these nonpermissive virogenic mammalian cells with permissive chicken cells. [The *SCI*® indicates that this paper has been cited in more than 155 publications.]

Oncogenic Provirus Integration and Rescue

Jan Svoboda
Institute of Molecular Genetics
Czechoslovak Academy of Sciences
166 37 Prague 6
Czechoslovakia

September 24, 1990

As a research fellow of the Institute of Experimental Biology and Genetics directed by Milan Hašek I proceeded with my studies of the rat XC tumor, harboring the chicken Rous sarcoma virus (RSV) genome. From the standpoint of outside observers, this model gave an impression of an experimental artifact and, not surprisingly, aroused little interest at the institute. I remember the director's repeated comments: What else can you do with that and when are you going to stop it? I argued that the viral genome in XC cells should be responsible for the genetic change that transformed a fibroblast into a tumor cell, and therefore elucidation of the nature of the viral genome in XC cells might provide information about the mechanism of this change. This argument worked because of broadmindedness and a sense for new approaches to experimental genetics, characteristic features of Hašek's creative mind.

At an oncology conference in Bratislava, I attracted the attention of Dušan Šimkovič and Viliam Thurzo, director, Cancer Research Institute. We set up a real collaboration that included, on my side, overnight trips from Prague to Bratislava and transportation of even a microscope and micropipettes for cloning: The results of previous *in vivo* experiments were reproduced also in tissue culture, and we demonstrated that every monocellular XC clone retained the viral genome.[1] Ultracentrifugation separation of subcellular structures performed with the expertise of Ivan Hilgert conclusively demonstrated that no infectious virus was detectable in any subcellular fraction of 36-gram amounts of XC tumor or in large volumes of culture fluid. These results were corroborated also by serological analysis carried out largely by my PhD student Pavel Chyle.

On the basis of all the previous and present results, we called the XC cells virogenic cells and concluded that they harbored provirus that was regularly transmitted as a component of the cellular genome. This interpretation had been put forward independently of Howard Temin's postulation of provirus based on other experimental approaches.[2]

We also proposed and later showed, together with my PhD students Ivo Hlozánek and Oldřich Machala, that intact XC cells released virus as a result of complementation by fusion with chicken cells.[3] A similar finding was made independently by Philippe Vigier.[4]

XC cells became instrumental in providing final proof that the complete provirus was represented by DNA. On the occasion of a Czechoslovak Biological Society meeting in Brno in 1966, my student Tamara Rakušanová[5] presented DNA hybridization data supportive of the possibility of DNA provirus presence in XC cells. In the discussion about DNA transfer initiated by Miroslav Hill, it was agreed to combine the expertise of his laboratory in DNA uptake by chicken cells with our experience with virogenic cells and virus rescue. After the Warsaw Pact invasion of Czechoslovakia in 1968, the Hills, not surprisingly, decided to stay for good in France, our groups split up, and the Hills published the first positive results with XC DNA transfection,[6] which was quickly followed by our report.[7] From this source we have recently isolated a proviral structure corresponding to reverse transcribed *src* mRNA and have shown that it can, by recombination, again acquire retroviral gene sequences.[8]

XC cells were widely employed to provide evidence that retroviruses produce syncytia after infecting a proper type of cell.[9] This phenomenon was discovered by my former student Václav Klement during his stay in Bob Huebner's laboratory at the NIH in Bethesda, Maryland, while studying the possibility of RSV rescue by superinfection of XC cells with mammalian retroviruses. This led to the establishment of a fast test for murine leukemia viruses employed throughout the world. A similar test has been developed for HTLV and HIV. It is beyond the scope of this commentary to discuss the impact of the experience with transformation of mammalian cells with chicken retroviruses on HIV studies, but it at least provided a warning that retroviruses do not respect class barriers.

1. **Šimkovič D, Svoboda J & Valentova N.** Clonal analysis of line XC$_{TC}$ rat tumour cells (derived from tumour XC) grown in vitro. *Folia Biol. Prague* 9:82-91, 1963. (Cited 40 times.)
2. **Temin H M.** The DNA provirus hypothesis. *Science* 192:1075-80, 1976. (Cited 80 times.)
3. **Svoboda J, Machala O & Hlozánek I.** Influence of Sendai virus on RSV formation in mixed culture of virogenic mammalian cells and chicken fibroblasts. *Folia Biol. Prague* 13:155-7, 1967. (Cited 65 times.)
4. **Vigier P.** Persistence du génome du virus de Rous dans des cellules du hamster converties in vitro, et action du virus Sendai inactivé sur sa transmission aux cellules de poule (Persistence of the Rous virus genome in converted hamster cells in vitro, on the actions of inactivated Sendai virus through its transmission to chicken cells). *C. R. Hebd. Séances Acad. Sci. Sér. D* 264:422-5, 1967. (Cited 55 times.)
5. **Rakušanová T.** Use of nucleic acid hybridization method for the demonstration of presence of Rous sarcoma virus genome in transformed cells. (Nečas O & Dvořák M, eds.) *Cell differentiation*. Brno, Czechoslovakia: Purkyně, 1967. p. 43-4.
6. **Hill M & Hillová J.** Production virale dans les fibroblastes de poule traités par l'acide désoxyribonucléique de cellules XC de rat transformées par le virus de Rous (Viral production in chicken fibroblasts treated with desoxyribonucleic acid from XC cells of the rat transformed by the Rous virus). *C. R. Hebd. Séances Acad. Sci. Sér. D* 272:3094-7, 1971. (Cited 85 times.)
7. **Svoboda J, Hlozanek I & Mach O.** Detection of chicken sarcoma virus after transfection of chicken fibroblasts with DNA isolated from mammalian cells transformed with Rous virus. *Folia Biol. Prague* 18:149-53, 1972. (Cited 50 times.)
8. **Svoboda J, Kandala J C, Geryk J, Pichrtová J & Guntaka R V.** A transformation-competent recombinant between v-*src* and Rous-associated virus RAV-1. *J. Virol.* 64:1873-7, 1990.
9. **Klement V, Rowe W P, Hartley J W & Pugh W E.** Mixed culture cytopathogenicity: a new test for growth of murine leukemia viruses in tissue culture. *Proc. Nat. Acad. Sci. USA* 63:753-8, 1969. (Cited 325 times.)

IA-18