p53 and chemosensitivity

To the editor — Wahl *et al.*[1] found that loss of wild-type p53-sensitized mammalian cells to Taxol cytotoxicity. They demonstrated that normal primary human lung fibroblasts, mouse embryo fibroblasts and human foreskin fibroblasts became more sensitive to Taxol if they lacked p53, either through targeted disruption of the p53 gene, targeted degradation using HPV16 E6 or functional inactivation using SV40 large T-antigen. Increased sensitivity to Taxol correlated with increased G2/M cell-cycle arrest and apoptosis induction in cells lacking functional p53.

We have been studying the role of p53 in determining the chemosensitivity of human cancer cells from different tissues to the clinically useful agents adriamycin, carboplatinum, cytoxan, and etoposide[2]. We found that endogenous p53 status predicted *in vitro* chemosensitivity in only a minority of cancer cell lines (ovarian and some Burkitt's lymphoma cells, but not in leukemia or lung cancer cells). We used HPV16 E6 to target degradation of p53, in wild-type p53-expressing H460 human lung cancer cells, via ubiquitin-mediated proteolysis. The H460 lung cancer cells did not become more resistant to adriamycin or etoposide when their p53 was degraded[2].

We have since engineered PA-1 human ovarian teratocarcinoma cells to express HPV16 E6 protein, since our initial observations suggested that, in these cells, p53 status may in fact be an important determinant of chemosensitivity. Fig. 1 shows that p53 protein was undetectable either before or after chemotherapy in two independent PA-1/E6 clones, and that induction of $p21^{WAF1/CIP1}$, the cell-cycle inhibitor, by chemotherapy was also lost in the E6-transfected cells as compared with either parental cells or a neo-clone. As previously demonstrated with H460-E6 cells[2], the E6-transfected ovarian carcinoma clones also displayed a deficiency in cell-cycle arrest following exposure to etoposide (data not shown), thus confirming loss of p53-checkpoint function. We confirmed that p53 mRNA was produced at similar levels in

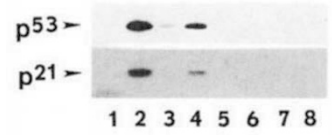

Fig. 1 Disruption of p53 function in human ovarian teratocarcinoma (PA-1) cells. Western blot analysis of p53 (upper panel) and p21 (lower panel) protein expression in PA-1 cells (lanes 1, 2), a G418-resistant clone (PA-1/Neo; lanes 3, 4) and two HPV16 E6-transfected clones (PA-1/E6-5, lanes 5, 6; and PA-1/E6-10, lanes 7, 8) is shown either untreated (lanes 1, 3, 5, 7) or treated (lanes 2, 4, 6, 8) with 0.2 μg/ml adriamycin as previously described[2]. p53 and p21 protein bands are indicated by arrows.

The true Western diet

To the editor — While being satisfied that Frederich *et al.* have validly proven the point expressed in their title (Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. *Nature Med.* **1**, 1311–1314; 1995), I confess to being somewhat disturbed by some implications and imprecisions in the paper itself.

First, they give the name "Western" to the experimental diet they fed their mice. Of course, Messrs. Teklad are free to call their wares by whatever name they chose, but the obvious, and possibly misleading, implication is that this mouse diet has something to do with anything typically consumed by humans in Western countries.

Yet the diet fed to these mice misses the mark by a wide margin. Admittedly, the French use butter freely (as other Western nations use olive oil or animal fat), but the proportion of fat (21% wt/wt) in the experimental diet reminds one of the regimen of the Inuits, who are Western only because they are so far east. More important, in my opinion, is the fact that, of the nearly 50% (wt/wt) of carbohydrates in the diet, 70% was sucrose, whereas in Western Europe the bulk of carbohydrates is provided by starch, under the guise of bread, pasta or potatoes: more accurately than "high fat," the diet fed the experimental mice should be called "high sucrose." Finally, cellulose appears to be absent from the mouse diet, whereas, as vegetables, it is an important constituent of meals eaten in Western Europe.

The above considerations reflect on a possible "Western is bad" indiscriminate interpretation of the Frederich *et al.* results by the general public. More relevant to their scientific interpretation might be the following. The experiments were performed under an *ad libitum* protocol. Ingested quantities would be central in the interpretation of results. (In fact, the authors only indicate quantities ingested in the final week of the experiment and these are not significantly different between groups.) The data provided do not afford the elements that could be used to falsify an alternative hypothesis to the "high-fat diet" one. Sucrose, being more rewarding a food than is standard Mouse Chow, induces, when given immediately after weaning, a feeding behavior leading to obesity. Increased body fat then induces increased serum leptin levels, which reduces food intake to normal, but is insufficient to reduce body fat. This interpretation, instead of being static, is a sequential one. It would fit data, too numerous to be cited, that show that "normal" obese humans have a normal or quasi-normal food intake, and that weight reduction necessitates a drastic reduction of food intake to below normal.

Of course, I am writing all this because I have been fed by my mother and then by my wife a perfect "Western" diet, complete with Roti Sauce Béarnaise and Sauerkraut Garni and Homard à l'Américaine and Pasta Fagioli, washed down with appropriate quantities of French wines, without ever any idea of "dieting." I am 67 years old, 1.72 m tall and weigh 64 kg, and am in apparently perfect health. Are genes everything, as suggested by my svelte parents who lived respectively to ages 91 and 89, and *a contrario* by the UCP-DTA transgenic mice? Or do I owe a good response to leptin action to the fact that my mother never fed me sweets?

J.-F. FONCIN
Laboratoire de Neurohistologie
Laboratoire Montyon – La Salpêtrière
47 Boulevard de l'Hôpital
75651 Paris, Cedex 13, France

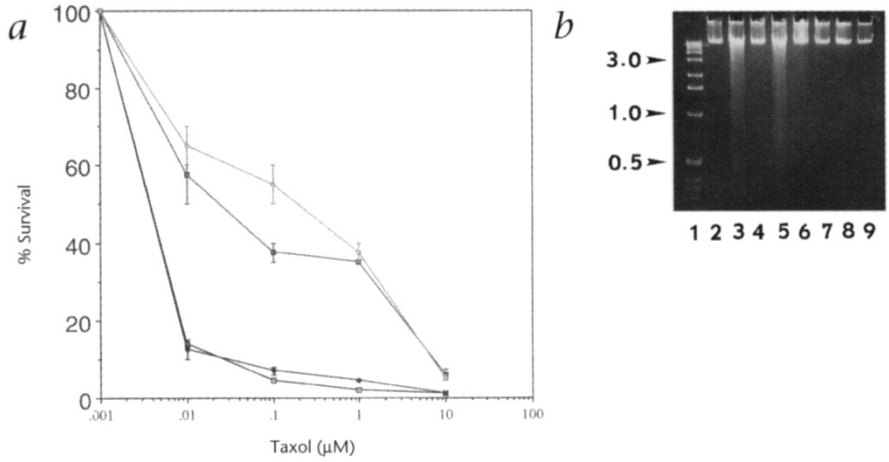

Fig. 2 Loss of p53 leads to Taxol resistance in teratocarcinoma cells. *a*, %-Clonogenic survival of parental PA-1 cells (□), G418-resistant PA-1/Neo cells (◆) and the two E6-transfected clones PA-1/E6-5 (■) and PA-1/E6-10 (◈) is shown as a function of Taxol dose. Clonogenic survival was determined as previously described[2]. *b*, Integrity of genomic DNA derived from parental PA-1 cells (lanes 2, 3), PA-1/Neo cells (lanes 4, 5), PA-1/E6-5 cells (lanes 6, 7) and PA-1/E6-10 cells (lanes 8, 9) is shown either in the absence (lanes 2, 4, 6, 8) or presence (lanes 3, 5, 7, 9) of 0.3 μg/ml adriamycin for 48 h. Isolation of genomic DNA was performed as previously described[3]. DNA size markers (in kilobases) are indicated by arrows corresponding to appropriate bands on a "1.0-kb ladder" (lane 1).

parental, neo and E6-transfected cells (data not shown).

We checked the chemosensitivity of the E6-expressing clones as compared with parental or a neo-clone and found that they not only became more resistant to adriamycin, carboplatinum and etoposide (not shown), but surprisingly (given the recent report by Wahl *et al.*[1]) also to the antimicrotubule agent Taxol (Fig. 2*a*). These ovarian teratocarcinoma cells became more than 100 times as resistant to Taxol following targeted degradation of wild-type p53 through expression of E6. We also checked the E6-expressing H460 lung cancer cells[2] for their sensitivity to Taxol, and again surprisingly did not observe a two log enhancement in chemosensitivity as has been reported by Wahl *et al.*[1] for normal fibroblasts lacking wild-type p53 function (data not shown). We correlated the increased resistance of the E6-expressing ovarian carcinoma clones with decreased apoptosis induction following chemotherapy (Fig. 2*b*). Endonucleolytic cleavage of DNA was only observed in PA-1 parental cells (Fig. 2*b* lane 3) or G418-resistant PA-1 cells (Fig. 2*b* lane 5) but not in E6-transfected clones (lanes 7 and 9) following exposure to chemotherapy.

Our observations suggest that there may not only be tissue-specific differences in the relationship between p53 status and chemosensitivity[2], but there may be a difference between loss of wild-type p53 function in normal fibroblasts versus cancer cells. It is not therefore obvious that the acquisition of p53 mutations associated with cancer progression would have predictable consequences with respect to sensitivity to Taxol therapy. It may be of interest to study the effects of p53 loss in the normal precursors of the ovarian teratocarcinoma cells. It is clear that disruption of wild-type p53 function by HPV16 E6 did not increase Taxol sensitivity in PA-1 ovarian teratocarcinoma cells. More work needs to be done to better understand these relationships in ovarian as well as other cancer cells.

GEN SHENG WU & WAFIK S. EL-DEIRY
Howard Hughes Medical Institute
Department of Medicine and Genetics
University of Pennsylvania
School of Medicine
415 Curie Boulevard, CRB 437
Philadelphia, Pennsylvania 19104, USA

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2. Wu, G.S. & El-Deiry, W.S. Apoptotic death of tumor cells correlates with chemosensitivity, independent of p53 or Bcl2. *Clin. Cancer Res.* (in the press).
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Edward Jenner 200 years on

To the editor — At a time when infectious diseases are in the headlines and continue to threaten mankind, there is a bicentennial that, surprisingly, is being forgotten, yet lends itself to some reflection.

On 14 May 1796 Edward Jenner injected an 8-year-old boy, James Phipps, with the pustules of cows harboring a disease similar to smallpox, the first vaccine. This event occurred after a cultural debate that spanned the whole eighteenth century. Now, 200 years and a worldwide effort have eradicated small pox.

It is interesting to note that the triumph of the smallpox vaccine occurred in the absence of a sound theory of immunity. It was based on careful epidemiological weighing of the risk-to-benefit ratio of vaccination. This should prompt some thought in those who view progress in science as founded mainly on formulation and disproving of general hypotheses. It is also interesting that at the time of Jenner's experiment, the social soil was most apt to accept vaccination. Years of debate had involved the most vivid intellects of the century (Voltaire in France, Beccaria and Parini in Italy), as well as politicians and royalty. Of course, the way vaccination was introduced and practiced in the eighteenth century can be criticized (orphans and slaves were widely used for experimentation); however, all in all, a social attitude must underlie the development of vaccines and the accompanying political debate. Molecular biology and immunology now offer potent conceptual and molecular tools, but are we, as industrialized societies, ready to invest a decent amount of financial and intellectual resources in tackling the infectious diseases that still plague most of the world? Social concern was, and still should be, an integral component of medicine.

Finally, Jenner's paper was rejected by the Royal Society and published at his own expense. Rejection of Jenner's paper by the most prestigious forum of the time should serve as a warning against excessive fetishism surrounding journal impact factors and other citation measures.

ALBERTO MANTOVANI
Istituto di Ricerche Farmacologiche "Mario Negri"
Laboratory of Immunology
Via Eritrea 62, 20157 Milan, Italy
University of Brescia, 25123 Brescia, Italy