Commentary on Eagle and Foley: "Cytotoxicity in Human Cell Cultures"

Michael B. Sporn

See related article by Eagle and Foley, Cancer Res 1958;18: 1017–25.

From a contemporary perspective in 2016, it is difficult to realize that not very long ago, the prevailing primary screen for testing new anticancer drugs was the demonstration of their therapeutic activity *in vivo* in animal models. Such assays were performed in mice that had been previously transplanted with highly malignant allogeneic or isogeneic carcinomas, leukemias, or sarcomas, which resulted in the subsequent death of the host mice if they were left untreated. The ability of a new drug to extend time to death was used as an endpoint of desired therapeutic activity.

Although these *in vivo* assays were cumbersome and expensive, and also required relatively large amounts of any new drug (as it was necessary to treat the mice repeatedly over many days), they were widely used and yielded results of major significance in cancer research performed in the 1940s and 1950s. Thus, the groundbreaking investigations by Hitchings and Elion on the synthesis of new purine analogues, which led to the eventual clinical use of 6-mercaptopurine as a component of life-saving leukemia therapy, relied on *in vivo* testing of newly synthesized purine analogues to evaluate their potential value as new drugs (1).

However, as efforts to synthesize new anticancer drugs increased markedly in the 1950s, it became apparent that a more efficient and less costly primary screen of new compounds was critically needed. The publication of Eagle and Foley's article in *Cancer Research* (2) in 1958 thus was of major importance as a definitive statement of the utility of cell culture technology for evaluating new drugs. In this landmark article, 180 compounds, which had previously been tested *in vivo* for therapeutic activity against at least three transplanted tumors, were systematically evaluated for cytotoxicity in two human cell cultures derived from either malignant or normal tissues. The human cells used were the malignant KB line, derived by Eagle from an epidermoid carcinoma of the nasopharynx (3), and a nonmalignant cell strain from normal liver, recently established by Chang (4). The known anticancer activity *in vivo* of the 180 compounds ranged from highly active to essentially inactive.

The results reported in Eagle and Foley's cell culture studies showed that the compounds that had been known to be active *in vivo* were also highly cytotoxic to both KB cells and Chang liver cells in culture. Cytotoxicity was essentially equivalent in both KB and Chang liver cells. Furthermore, compounds that were inactive drugs *in vivo* were largely inactive in cell culture; false positives were relatively rare, as only 19 of 91 (21%) compounds, inactive *in vivo*, were found to be cytotoxic to the cell cultures.

This landmark study, which turned out to be so influential in directing the course of future screening of anticancer drugs, was the culmination of many years of meticulous research in the Eagle and Foley laboratories. These previous studies had delineated the specific amino acid, salt, vitamin, and serum requirements for tissue culture of human and mouse cells (5, 6), leading to the formulations what are now known as the various forms of "Eagle's Medium." The development of defined media for growing cells in monolayer culture was a major advance.

The extensive study published by Eagle and Foley in *Cancer Research* in 1958 was actually an outgrowth of a seminal, but narrower, investigation they had published in 1956 in the *American Journal of Medicine* (7). In this latter article, they had shown that 13 drugs with known anticancer activity *in vivo* against transplanted tumors were also markedly cytotoxic in cell culture when tested against several tumor cell lines. The active compounds included amethopterin (methotrexate) and 6-mercaptopurine. In their 1956 summary, Eagle and Foley suggested that tissue culture methodology could be useful as a routine screening method for new anticancer drugs, and they then proceeded to perform the more extensive testing of the 180 compounds, which was published in 1958.

Eagle and Foley's approach to first-pass screening of new anticancer drugs in cell culture, as predicted, soon became an accepted modality, and it continues to be so to the present day. Clearly, we now realize that the overall context of cancer is much more complicated than the mere growth of isolated tumor cells and that the tumor microenvironment is an essential feature of any malignancy. The essential role of inflammatory, immune, and vascular cells in regulating the progression and metastasis of malignancy (8, 9) could barely be foreseen in 1958. Moreover, screening in cell culture also ignores the fundamental problem of tumor heterogeneity (10). In spite of these limitations, Eagle and Foley clearly foresaw a fundamental and critical problem that has continued to plague cancer chemotherapy right up to the present time. They made the important observation that active anticancer drugs also can cause equivalent cytotoxicity to nonmalignant, as well as malignant cells. Hopefully, new advances in developing targeted therapies that are not cytotoxic will eventually eliminate this problem altogether, but this is still a pressing issue in modern cancer chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received January 15, 2016; accepted January 15, 2016; published online March 1, 2016.

Department of Pharmacology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire.

Corresponding Author: Michael B. Sporn, The Geisel School of Medicine at Dartmouth, Hanover, NH 03755. E-mail: Michael.Sporn@Dartmouth.edu

doi: 10.1158/0008-5472.CAN-16-0152

References

1. Clarke DA, Philips FS, Sternberg SS, Stock CC, Elion GB, Hitchings GH. 6-Mercaptopurine: effects in mouse sarcoma 180 and in normal animals. Cancer Res 1953;13:593–604.
2. Eagle H, Foley GE. Cytotoxicity in human cell cultures as a primary screen for the detection of anti-tumor agents. Cancer Res 1958;18:1017–25.
3. Eagle H. Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. Proc Soc Exp Biol Med 1955;89:362–4.
4. Chang RS. Continuous subcultivation of epithelial-like cells from normal human tissues. Proc Soc Exp Biol Med 1954;87:440–3.
5. Eagle H. The specific amino acid requirements of a human carcinoma cell (strain HeLa) in tissue culture. J Exp Med 1955;102:37.
6. Eagle H. The specific amino acid requirements of a mammalian cell (strain L) in tissue culture. J Biol Chem 1955;214:839.
7. Eagle H, Foley GE. The cytotoxic action of carcinolytic agents in tissue culture. Am J Med 1956;21:739–49.
8. Albini A, Sporn MB. The tumor microenvironment as a target for chemoprevention. Nat Rev Cancer 2007;7:139–47.
9. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med 2011; 17:320–9.
10. Welch DR. Tumor heterogeneity—a contemporary concept founded on historical insights and predictions. Cancer Res 2016;76:4–6.