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Seminars in Cancer Biology 14 (2004) 449–452

seminars in
CANCER BIOLOGY

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Modern criteria to determine the etiology of human carcinogens

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Abstract

Rapid identification of human carcinogens before their dissemination into society, and exposure of worker and lay populations is an important goal of cancer research. Retroactively, verification of in-place human carcinogens is also required to target their removal, and other preventive and therapeutic strategies. The hierarchy of methods used historically for evaluation of carcinogenic potential is epidemiology > animal bioassays > mechanistic studies, and the focus has been on single agents that are genotoxic.

However, mechanistic research has revealed several obligatory steps in carcinogenesis, tumor promotion, and progression that can now be used in screening studies with human cells in vitro and animal bioassays. These approaches should be combined with molecular epidemiology and molecular pathology to identify human carcinogens with more emphasis on evaluating combinations of suspect agents and mechanisms of action of epigenetic carcinogens.

Keywords: Epidemiology; Animal bioassays; Molecular biology; Carcinogens; Classification

1. Introduction

Of the many needs in cancer research, the identification of putative human carcinogens and their inactivation or removal from society is an important priority for prevention of future cancer risks. Although individual human carcinogens have been classified historically based primarily upon their carcinogenicity alone and the results of epidemiology, animal, and mechanistic studies, scientific research over the past several decades has illustrated that agents other than classic initiators and/or genotoxic agents can act as co-carcinogens and substances which enhance the processes of tumor promotion, progression, and angiogenesis. These observations indicate the importance of mechanistic studies and evaluating interactions between agents in establishing their etiologies. In addition, analyses of individual agents may be misleading and insensitive if they cause tumors in small numbers of the human population or only in certain subgroups of individuals at high risk because of their genetic background or undefined environmental factors, including diet or exposure to co-carcinogens.

The most notorious example of a complex human carcinogen, which was not identified until well after widespread exposure occurred, is ‘asbestos’, a family of chemically and physically diverse, naturally occurring fibers that have been mined and used industrially for most of the 20th century [1]. Certain types of asbestos fibers with different pathogenic potential, i.e. the amphiboles versus serpentine chrysotile, occur in different geographic areas and/or in association with other types of fibers or co-carcinogens [2]. Although the association of amphibole asbestos with the development of mesothelioma was first made in 1960 by Wagner and colleagues in South Africa [3], the rare occurrence of this tumor in the US and other countries went unnoticed until the development of modern diagnostic pathology and mineralogic techniques for identification of asbestos fibers in the lung and pleura. The fact that asbestos and smoking were co-factors in lung cancer was recognized almost a decade later in a US cohort, but mechanistic studies at the time showed that asbestos failed to act as a genotoxic agent in lung epithelial cells (reviewed in [2]). The role of asbestos as a co-carcinogen, increasing the intracellular delivery of bioreactive polycyclic aromatic hydrocarbons (PAHs) [4], as well as a tumor promoter that causes hyperplasia and sqamous metaplasia of airway epithelium [5] confirmed that this ubiquitous carcinogen had multiple roles in cancer causation. Epidemiologic detection of increased cancer risks was further complicated by the fact that the average latency periods

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doi:10.1016/j.semcancer.2004.06.008

of asbestos-associated lung cancers and mesotheliomas (>35 years) are extremely protracted [6]. With billions of tons of asbestos in place in buildings, the removal of asbestos has been an arduous and expensive task, and the overall impact of this carcinogen has been devastating worldwide. Although scientists have been prudent in educating the public about the cancer risks and best policies for removal of asbestos in the last decades [2], it would have been desirable to identify the carcinogenicity of asbestos before its widespread use in industry and exposure of the general population.

In this chapter, we review the current criteria for identification of human carcinogens and the development of modern approaches for their more effective and rapid screening. Our chapter largely synthesizes the recent conclusions from a panel of experts in the field at a recent workshop designed to evaluate relevant and "state of the art" criteria to establish the etiology of human carcinogens [7].

2. Criteria for classification of human carcinogens should incorporate mechanistic information

The several agencies with published criteria for the identification of human carcinogens have similar approaches for evaluation, including review of epidemiologic studies, experimental studies in animals, and more recently, incorporation of other relevant data on carcinogenic mechanisms (see Chapter 1). All these approaches have some limitations, but the "gold standard" is usually epidemiology, which clearly finds associations, but cannot establish causality. Epidemiology, which classically has defined associations between workplace concentrations of carcinogens as opposed to lower levels that may be more relevant to societal exposures, is also relatively insensitive for these and other reasons, including its inability to delineate complex confounding variables. Animal bioassays are complementary to epidemiology in that they can control for confounding factors (if known), exposure routes, and dose–response concentrations of agents. However, species variables may exist, and some concerns have been expressed in that false-negatives and false-positives have been found when data are compared to humans. This conundrum can be addressed, in part, by mechanistic studies and demonstration that the mechanism is likely to occur in humans.

Excellent examples of the guidance of mechanistic information in reinforcing or negating the results of animal bioassays and epidemiology include the results of studies on dioxin and saccharin, respectively. In the case of dioxin, a multi-site carcinogen in animal studies, mechanistic work revealed that the chemical acted through the Ah receptor, which is highly conserved in evolution and functions similarly in humans and mammals. Moreover, the concentrations of dioxin causing cancer in rats were similar to those in heavily exposed human populations in which increases occurred in cancer risk. On the other hand, testing of saccharin in rats revealed the development of urinary bladder cancer which appeared to be related to the unique physiology of the rat urinary system and were only found when concentrations of saccharin in the rat diet were 3% or higher, a situation not plausible in humans.

Although mechanistic data have been considered important in supporting or weakening observations from epidemiologic and animal studies, modern mechanistic studies have identified many of the precursor and critical events in establishment of human cancers. Experiments using human and animal cells comparatively have been valuable in establishing which of these criteria are relevant to the establishment and maintenance of human neoplasms. Thus, it should be possible theoretically to identify or target carcinogens revealed by mechanistic studies as playing a role in tumor development in man, either alone or by interacting with other agents. Clearly, a human carcinogen as currently defined induces cancer, but the challenge is to also identify agents that play a role in tumor development or invasion by interacting with the complex internal mileau. Ideally, epidemiologic evidence, experimental evidence in animals, and mechanistic studies should reinforce each other in defining a human carcinogen, regardless of its potential multiplicity of roles in human carcinogenesis.

3. Challenges and rewards

There are several landmark mechanistic studies that have defined obligatory events in the development of cancer, including the inactivation of both Rb and p53 pathways. There are also numerous genetic markers and new "cancer genes" (and tumor suppressor genes) being identified on a regular basis. These may be globally important or reflective of species, cell type, or tissue-specific cancers. It is not unreasonable to expect that there might also be several "cancer susceptibility genes" or genes intrinsic to genetic instability that will be identified in the future. If this long "wish list" can be functionally reduced to key genes or genetic changes critical to human carcinogenesis, they can be used as predictive biomarkers of prevention in human populations.

Additional challenges in molecular epidemiologic studies, which have already successfully used DNA adducts, and mutated genes and proteins as markers of exposure in human populations, are to sort out other critical co-factors that contribute causally to cancer risk, especially since multiple chemical exposures and gene and virus–environmental interactions probably exist in susceptible human populations. Moreover, epigenetic alterations and mechanisms of action of epigenetic carcinogens that play a role in the development of human cancer, i.e. via dysregulation of methylation, genomic imprinting, etc., demand further attention in assays that will reveal and categorize these agents. For example, viral infections can be considered as epigenetic effects in some cancers.

Defining a human carcinogen also presents the additional problems of individual heterogeneity in response to geno-

toxic carcinogens and epigenetic factors, including differences in DNA repair and metabolism of carcinogens, dietary factors, and life styles. These additional variables may be difficult to mimic in mechanistic and animal studies, and make individual risk factors impossible to resolve in many epidemiologic studies. However, understanding the pathways of these variables, i.e. metabolic profiles, etc., and their species or individual differences may facilitate the development of rapid and accurate methodology for genotyping and phenotyping individuals for relative susceptibility to some cancers. One area that this may be applied successfully to is individual susceptibility to tobacco-induced cancer, of which there is a constantly increasing data base on variants of genes involved in carcinogen metabolism and DNA repair, etc. Since there are thousands of potential carcinogens and co-factors influencing human carcinogenesis, the development of single, rapid, and predictive in vitro tests for human carcinogens or susceptibility is daunting and unlikely. However, tests for agents inducing DNA mutations (the Ame's test, etc.) and the elucidation of key steps in the processes of tumor promotion, progression, and metastases may someday yield a battery of parameters that can be exploited in the identification and classification of human carcinogens. The "omics" revolution will undoubtedly aid in defining these strategies and lead to a better definition of biomarkers of exposure to carcinogens while defining the critical processes more completely.

4. Critical questions and recommendations

Key important questions and suggestions to improve upon the current criteria for the validation of a human carcinogen are presented below.

4.1. Should the criteria for classification of a human carcinogen be the same for different carcinogenic agents (i.e. viruses, chemicals, etc.)?

One might argue that the current critera (epidemiology, animal bioassays, and mechanistic studies in a supportive role) should remain unaltered because we lack evidence currently to support a more accurate classification schema. Modern epidemiologic studies that now depend upon genetic, biochemical, and molecular techniques not available when the Hill's criteria to identify environmental carcinogens were advocated in the 1970s, will continue to improve the accuracy of classification of different types of carcinogens in general via increased sensitivity and precision (see Chapter 2). However, more attention should be given to classification of biological agents, such as viruses, based upon their disparate mechanisms of action. One suggestion is that biologic carcinogens, i.e. microbial agents and viruses, might be classified as "direct carcinogens" versus "indirect carcinogens". The former could be classified based upon: (1) demonstration of the regular presence of part or all of its genome in every cancer cell; (2) excision of its nucleic acid from a transfected or cancer cell harboring its DNA or RNA; (3) demonstration that inhibition of the function of its nucleic acid, i.e. use of RNA interference or antisense technology, leads to a reversion of an immortalized or malignant phenotype in infected cells; and (4) transfection of its nucleic acid into cells in culture or laboratory animals results in cell immortalization or tumor development, respectively. In the definition of an "indirect carcinogen", clinical/epidemiologic observations and animal/in vitro studies should point to the role of the agent as a co-carcinogenic factor. Regardless, epidemiologic studies should contribute to the observations that agents are major risk factors or co-factors in human cancer risk, and in vitro evidence alone should not be considered proof of carcinogenicity.

4.2. If we can define the genetic/epigenetic changes that occur in cancer, how can this information be used to identify human carcinogens causing these phenomena?

Although the genetic/epigenetic changes that occur in some human cancers have been characterized using modern molecular pathology, this information is incomplete. Work thus far suggests that the genetic and epigenetic changes caused by chemical carcinogens are not specific. For example, we cannot link the majority of carcinogens to a specific genetic alteration. Moreover, the same carcinogen may induce multiple genetic alterations in a cell-type or tissue-specific fashion. Clearly, more research on expression and activation of cancer genes is needed to develop a panel of genetic markers for cancer as well as documentation of their presence in premalignant tissues. A strictly genetic tumor progression model is unproven in human cancers, emphasizing the need for information as well on epigenetic changes in the development of individual cancers and in response to specific exposures to carcinogens.

Knowing that a given agent alters key cellular mechanisms required for carcinogenesis, i.e. inactivation of Rb/p53, activation of *ras*, telomerase, invasion, and metastasis in human cells, together with positive data from animal studies affecting the same target organ, should be considered as a strong inference that the agent is a human carcinogen.

4.3. What are the promising developments and potential for using in vitro studies with animal bioassays to identify human carcinogens?

With the new technology available to culture differentiated human cells for prolonged periods of time and knowledge of critical mechanisms of carcinogenesis, including mutational spectra, in vitro studies can be used for initial screening of human carcinogens. It is critical that potential carcinogens be evaluated in human cells of the specific target organ, where tumors of similar histotypes develop in animal bioassays or man. Moreover, it is important to demonstrate that similar patterns of metabolism or uptake of carcinogens

occur in vitro and in vivo. Concentrations relevant to human exposures are difficult parameters to validate in in vitro studies, but dose–response studies with suspect agents can be performed less expensively and may be informative.

Several advances might increase the predictive value of in vitro studies and their overall utility. Based on mechanistic studies, we can now extend and embellish upon the current battery of in vitro tests for stages of transformation, mutagenesis, cell proliferation, invasion/migration, and angiogenesis. More attention is needed for the development of assays for critical steps in tumor promotion and progression (genetic instability, etc.). Once these assays are validated with known carcinogens, we can establish a data base for known carcinogens of different types that can be used in profiling suspect agents. Additional studies should explore the ramifications in these assays of using complex mixtures and combined exposures to genotoxic and epigenetic carcinogens. Information gleaned from profiling of critical genes and proteins using microarrays and proteomics will undoubtedly improve our mechanistic knowledge of carcinogenesis and allow validation of these macromolecules in human tissues via molecular pathology. Lastly, transgenic animal models and RNA interference approaches are powerful new tools that can be incorporated into strategies for carcinogen classification based on mechanistic approaches.

In conclusion, this chapter emphasizes the use of critical new data on mechanisms of carcinogenesis which can be successfully incorporated into both the testing of and rationale for classification of human carcinogens. Definition of obligatory steps in the carcinogenic process, invasion, and angiogenesis now provides scientists with additional data to make informative decisions that are bolstered by animal bioassays and modern epidemiologic approaches. Although more research is necessary, especially to define pathways of epigenetic carcinogens in cancer development, the rules for classifying many human carcinogens are being continually embellished by sound mechanistic science.

Acknowledgements

We thank the participants and panel members at the workshop on “Validation of a Causal Relationship: Criteria to Establish Etiology” sponsored by the Division of Cancer Biology of the National Cancer Institute in Washington, DC, on December 11–12, 2003, for helpful comments and opinions.

References

[1] Alleman JE, Mossman BT. Asbestos revisited. Sci Am 1997;70–5.
[2] Mossman BT, Bignon J, Corn M, Seaton A, Gee JBL. Asbestos: scientific developments and implications for public policy. Science 1990;247:294–301.
[3] Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960;17:260–71.
[4] Eastman A, Mossman BT, Bresnick E. Influence of asbestos on uptake and alkylation to DNA of benzo(a)pyrene in hamster tracheal epithelial cells. Cancer Res 1983;43:1251–7.
[5] Mossman BT, Craighead JE, MacPherson BV. Asbestos-induced epithelial changes in organ cultures of hamster trachea: inhibition by retinyl methyl ether. Science 1980;207:311–3.
[6] Mossman BT, Gee JBL. Medical Progress: asbestos-related diseases. N Engl J Med 1989;320:1721–30.
[7] Workshop on “Validation of a Causal Relationship: Criteria to Establish Etiology”. Division of Cancer Biology of the National Cancer Institute. Washington, DC; December 11–12, 2003.