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PROSPECT

Simian Virus 40 and Human Tumors: It Is Time to Study Mechanisms

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Abstract Several studies found simian virus 40 (SV40) in 47% to 83% of human mesotheliomas. Mesotheliomas are malignant tumors of the pleura and peritoneum, firmly associated with asbestos exposure. In this issue, Gazdar and colleagues [Shivapurkar et al., 1999] found that SV40 is present only in the malignant cells and not in the surrounding stromal cells. Using the microdissection technique, they found SV40 in 54% of 93 mesotheliomas of the epithelial type. The surrounding reactive stromal cells, (20 lung cancers and 14 mesotheliomas of the sarcomatoid/fibrous type) did not contain SV40, confirming the specificity of their positive findings. Furthermore, SV40 was found in 14% of 14 non-malignant reactive mesothelial cell proliferations. In 12 cases of mesothelioma a noninvasive (or in situ) component was also identified. In all four cases in which SV40 sequences were present in the invasive component, sequences were also present in the accompanying noninvasive component. These data suggest that the virus resides in the mesothelial cells prior to tumor development. The data address the remaining concerns raised at an International Meeting organized by the NIH, FDA, and CDC in 1997 to definitively associate SV40 with human mesothelioma. It is time now to investigate the pathogenic mechanisms of this association, and if SV40-infected mesothelial cells are more susceptible to other carcinogens, such as asbestos. Furthermore, we must investigate the interaction between the host immune system and SV40-infected mesothelial cells, and study if the immunosuppressive activity of asbestos interferes with tumor rejection. These studies should lead to a better understanding of mesothelioma pathogenesis, and possibly to new therapeutic approaches aimed at interfering with the expression of the SV40 genome and/or at eliciting a strong immune response against SV40 infected mesothelial cells. J. Cell. Biochem. 76:189–193, 1999.

Key words: SV40; asbestos; mesothelioma

In January of 1997 the NIH, the FDA, and the CDC organized an international meeting in Bethesda, Maryland, where leading virologists, molecular biologists, and physicians discussed the association of SV40 with human mesotheliomas, osteosarcomas, ependymomas, and other brain tumors [reviewed in Carbone et al., 1997a]. In that meeting, several laboratories confirmed that SV40 was present in mesotheliomas, ependymomas, and osteosarcomas. One study contracted by a group of epidemiologists to a virology laboratory reported negative findings. There was no explanation for the negative findings, except for the suggestion that the methodology used was not sufficiently sensitive to detect SV40. However, skepticism arose in the scientific community due to this single negative result. Concerns were also expressed that because infectious SV40 had been injected into humans through contaminated poliovaccines from 1955 until 1963, these results could scare the general public and discourage some people from vaccinating their children. To complicate the matter even further, the agencies responsible for the production and the distribution of the poliovaccines, as well as the insurance companies representing these agencies, were concerned about possible legal liability that had already been threatened in the lay press. Because of these concerns, a prudent approach appeared the best choice. It was agreed that before stating that SV40 was a human carcinogen present in certain types of human cancers the following had to be demonstrated: 1) that SV40 was reproducibly detectable in human tumors by a blind multilaboratory study organized by an independent and reputable organi-

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zation; 2) that SV40 was shown to be present in the tumor cells and not in the stromal cells by additional methods than immunohistochemistry; and 3) before making a possible link with causation, that the presence of SV40 was significantly higher in mesotheliomas (or in osteosarcomas and ependymomas) compared to other tumor types and/or normal tissue.

To address the first issue, the International Mesothelioma Interest Group, which is composed of the leading researchers in the field of mesothelioma, organized a multilaboratory study that was led by J.R. Testa of the Fox Chase Cancer Center. Following guidelines for the study proposed by P. Minor of the National Institute for Biological Standards in the UK, 12 mesotheliomas were investigated. Ten of 12 (83%) reproducibly tested positive for SV40 [Testa et al., 1998]. To contain costs for a study that had no specific funding, this study only addressed the question "Is SV40 present in mesotheliomas?" Other tumor types or normal tissue were not studied. Testa's study was important, because it provided an independent verification about the presence of SV40 in human mesotheliomas, and four independent laboratories confirmed the reproducibility of the findings. At the same time, this study had not been designed to investigate if SV40 was present only in malignant mesothelioma, or whether it was also present in the surrounding stroma and/or in other tumor types.

In the paper presented in this issue, A.F. Gazdar and colleagues [Shivapurkar et al., 1999] tested malignant mesothelioma cells and the nearby reactive stromal cells for SV40 using the microdissection technique. They also tested normal reactive mesothelium and lung cancer specimens for SV40. They analyzed 118 mesotheliomas, 14 normal reactive pleural effusions, and 20 lung cancers. The microdissection experiments demonstrated that SV40 was present only in mesothelioma cells and not in the nearby reactive stromal cells. This finding demonstrated the specificity of the association of SV40 with mesothelioma. These results also ruled out any concern about "PCR contamination," because it is impossible to specifically and reproducibly contaminate only the "mesothelial cells" when microdissecting the same sample for normal and malignant cells. Fifty-four percent of mesotheliomas of the epithelial type contained SV40 DNA sequences compared to 14% of SV40-positive "normal" reactive mesothelium (which is also of the "epithelial-type"). The difference was significant ($P = 0.01$). It should be noted that the majority of mesotheliomas have an epithelioid morphology (epithelial-type mesotheliomas), a few have a spindle cell morphology (sarcomatoid- or fibrous-type mesotheliomas), which is associated with a very aggressive tumor phenotype, and some have both an epithelioid and a spindle cell component (mixed-type mesotheliomas). Galateau-Sallé et al. [1998] found SV40 in 47.6% of mesotheliomas and in 16% of normal reactive mesothelium. We found SV40 in 60% of mesotheliomas [Carbone et al., 1994], and Cristaudo et al. [in press] found SV40 in 55.5% of mesotheliomas; Xu et al. [1999] found SV40 in 5% of cell lines derived from non-neoplastic mesothelium. Therefore, 5% to 16% of "normal" people may have SV40 in their mesothelial cells, compared to 47.6% to 83% of mesotheliomas. Furthermore, in Gazdar's study, SV40 was detected in both the preinvasive and the invasive component of mesotheliomas. These data, together, indicate that SV40 infects mesothelial cells before malignant transformation and suggest that SV40-positive individuals have a higher risk of developing mesothelioma. Twenty lung cancers and 14 sarcomatoid (fibrous) mesotheliomas tested negative for SV40, further confirming the specificity of the positive results.

Gazdar's study also addressed some additional concerns that had been raised at the Bethesda meeting in 1997. Some had expressed the concern that H.I. Pass, who had provided us with the mesothelioma specimens [Carbone et al., 1994], and/or his surgery room might have been contaminated with SV40. In Gazdar's study, mesothelioma specimens from different parts of the world were compared, including specimens from H.I. Pass. No differences were noted. Furthermore, among the specimens provided by H.I. Pass, only 1/20 adjacent (to the mesothelioma) lung specimens contained SV40 sequences, providing additional evidence that his specimens were not contaminated with SV40, and that SV40 was specifically associated with mesothelioma. It had also been suggested that the commercial reagents were contaminated with SV40; this also can be ruled out, otherwise no differences would have been noted in this and other studies, and all of the samples should have tested uniformly positive.

Finally, it was suggested that the laboratories were contaminated with plasmids containing SV40. This study also rules out this unlikely possibility, because if there had been a widespread SV40 contamination, the samples should have tested either uniformly positive, or scattered positive samples should have been detected randomly in the various types of specimens analyzed. Instead, Gazdar and colleagues found striking differences in positivity among mesotheliomas and other samples, which rules out background contamination. Even more convincing was the demonstration that SV40 was detectable only in the microdissected tumor cells and not in nearby microdissected stromal cells. It should also be noted that SV40 has been detected in human tumors using a variety of techniques which, in addition to the PCR, include: Southern blotting, mRNA in situ hybridization, Western blotting and immunostaining, [see for example, Martini et al., 1996; Carbone et al., 1997b, De Luca et al., 1997; Mendoza et al., 1998]. Infectious SV40 has also been rescued from human ependymomas [Lednicky et al., 1995]. Since these techniques are not PCR, the positive results obtained cannot be ascribed to PCR contamination. In conclusion, the paper in this issue confirms the presence of SV40 in mesotheliomas; confirms the reliability of PCR to demonstrate the presence of SV40 in mesotheliomas; demonstrates that SV40 is specifically present in malignant mesothelioma cells and not in nearby stromal cells; and finds that SV40 is present in the mesothelial cells of a small but substantial percentage (14%) of "normal" individuals. Therefore, this paper addresses the remaining concerns about the association of SV40 with human mesothelioma.

Gazdar and colleagues found no significant differences in SV40 positivity in paraffin-embedded specimens compared to frozen specimens. They also state that the hypothesis that paraffin specimens are not suitable for SV40 detection is incorrect. We agree, because we routinely test paraffin specimens for SV40. However, we recommend the use of frozen tissue to those laboratories that are not devoted to these types of studies. Gazdar's group is one of the leading research teams in lung cancer, with extensive experience with PCR analyses of human tumors. Furthermore, they included A. Churg, Chairman of the US-Canadian Pathology Panel for Mesothelioma, on their team to identify the specimens, or the portions of the specimens, more suitable for microdissection and PCR analyses. Pepper et al. [1996], De Luca et al. [1997], Strizzi et al. [in press], and Cristaudo et al. [in press] who had similar research settings, and included leading mesothelioma pathologists in their teams, also had no difficulty detecting SV40 in paraffin specimens. On the other hand, laboratories having no experience with mesothelioma specimens may not take the precautions required when handling mesothelioma biopsies—these biopsies are often very small and contain few tumor cells among a majority of reactive stromal cells—and may fail to detect SV40. This has happened in the past and has created an unnecessary controversy, which has delayed progress in this research field. Therefore, to diminish the risk of false negative results, I think that frozen biopsies may be a better choice when available, because they are bigger and contain no degraded DNA.

Now that the association of SV40 with mesothelioma is well established, it is time to move on. The higher percentage of SV40-positive mesothelioma samples compared to non-malignant mesothelial samples suggests that mesothelioma preferentially develops in SV40-positive mesothelial cells. SV40 is one of the most oncogenic tumor viruses, and is capable of causing mesothelioma in 100% of hamsters in three to six months and of transforming human cells in tissue culture. SV40-transformed human cells grew as subcutaneous tumors when injected into human volunteers; however, these tumors eventually regressed because of immune rejection [Jensen et al., 1964]. Therefore, SV40 T-antigen (Tag, the SV40 oncogene) is a potent carcinogen, but it is also a strong immunogen. Thus, in human mesothelial cells, SV40 Tag should be able to first cause malignant transformation, and then induce tumor rejection. In mesotheliomas, SV40 Tag binds and inactivates cellular p53, pRb, p107 and p130/Rb2 [Carbone et al., 1997b; De Luca et al., 1997]; these data suggest that SV40 contributes to the transformed phenotype. However, why are SV40-positive tumor cells not rejected by the immune system? In fact, it could very well be that they are. It is possible that most mesothelial cells that express sufficient amounts of Tag are lysed by the immune cells. The well known local and systemic immunosuppressive

activity of asbestos [Rosenthal et al., 1999], a carcinogen firmly linked to mesothelioma [Robledo and Mossman 1999; Murthy and Testa, 1999], may, however, favor the development of mesothelioma. In addition, SV40-positive mesothelioma cells express very low levels of Tag [Carbone et al., 1997a]. These low levels are both sufficient and necessary to maintain the transformed phenotype [Waheed et al., in press], but may be below the threshold level of detection by the immune system. It is possible that only cells expressing low levels of Tag can escape the immune system, and that the immunosuppressive effects of asbestos may contribute to tumor growth. Future studies should investigate these possibilities. This knowledge would be very useful in designing new immunological and genetic approaches to treat SV40 positive mesotheliomas. Some of these approaches are being developed. Specifically, we are collaborating to bring the anti-SV40 vaccine designed by M.G. Sanda and colleagues to treat mesothelioma patients to phase 1 clinical trial [Xie et al., 1999]. D. Schrump and colleagues are pursuing an antisense strategy, because they have found that by downregulating the expression of Tag they can induce growth arrest and apoptosis in human malignant mesothelioma cell lines positive for SV40 [Waheed et al., in press]. These are very exciting developments and bring some hope for SV40-positive mesothelioma patients who are presently faced with a median survival of one year from diagnosis. Therefore, it is important to study both the mechanisms used by SV40 to transform mesothelial cells, and the interaction between SV40-transformed mesothelioma cells and the immune system. Furthermore, by studying the mechanism of interaction between asbestos and SV40, we may learn how viruses and environmental carcinogens interact to cause some human cancers.

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