Support for CIN 2-3 being an immediate result of infection by high-risk types of HPV comes from the observation in one study (8) that incident CIN 2-3 occurred shortly after detection of HPV 16 or 18 DNA among women who were cytologically negative at study entry. Furthermore, infection of human keratinocyte rafts with HPV 16 results in a morphologic lesion resembling CIN 2-3 (9), but infection by HPV 6 is more likely to result in a lesion resembling CIN 1. Whether the 20%-30% of CIN 1 lesions containing HPV 16 could progress to carcinoma, or whether the type of epithelium (mature versus immature squamous epithelium) is important in determining the natural history of infection with specific HPV types, requires further investigation. We must also reassess whether such findings are simply the result of small areas of CIN 2-3 missed on routine cytologic sampling because of size or location (e.g., within glands) or perhaps a slower rate of exfoliation of cells.

In short, studies such as that of Schiffman et al. have provided compelling data for viewing CIN as lesions directly resulting from cervical HPV infection and should lead us to reconsider the appropriateness of the importance we now assign those lesions caused by HPV types without apparent oncogenic potential. The natural history of HPV infection varies substantially. Factors that are important in determining the outcome of HPV cervical infections include HPV type or types present, location of the infected cells within the cervical epithelium, and host characteristics such as age and immunologic and hormonal status. Given what we know about the biology of HPV and the epidemiology of cervical cancer, it is not unreasonable now to undertake studies in a variety of different settings to test the hypothesis that HPV typing can be used to help decide which women with abnormal cytologic findings consistent with CIN 1 or less are in need of immediate treatment and which can be followed conservatively.

Limited Role of Vasculature-Mediated Injury in Tumor Response to Radiotherapy

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Much of cancer research is aimed at identifying the differences between normal and malignant cells so that a therapeutic window can be established, using an agent that will kill enough tumor cells to control the disease without causing excessive normal tissue damage. It has been recognized, however, that cells of both tumors and normal tissues can be killed by two mechanisms: 1) direct damage to the cells or 2) indirect damage to the cells resulting from injury to the vascular network. This vasculature-mediated injury has for many years been of particular interest to radiobiologists and radiotherapists, since it may be partly or totally responsible for late normal-tissue damage occurring many months after the treatment has been completed (1). Thus, an understanding of the factors controlling vascular radiosensitivity could allow us to reduce late complications of therapy.

The reason why late changes are seen after radiation therapy relates to the fact that DNA damage, although inflicted within microseconds by ionizing radiation, can remain latent in cells for months, not interfering with the normal biochemistry of the cells, but becoming evident only when the cells attempt to divide (2). Abortive mitoses lead to cellular depletion and to proliferative sterilization of the progenitors that should repopulate the damaged tissue or tumor. Vascular endothelial cells in all normal tissues have a very slow rate of proliferation, with a turnover time of months to years (3); hence, there should be a long latency before the abortive divisions take place and tissue dysfunction is recognized. Of course, if the parenchymal cells in the tissue are also slow growing, the direct radiation injury to...
those cells will also show a long latency, and careful, de-
tailed studies are required to distinguish direct cell kill from
injury mediated through damage to the vasculature (4).

In 1982, it was recognized that endothelial cell prolifera-
tion in tumors is very much faster than in normal tissues (5),
and these cells were identified as a potential new target for
therapy (6,7). Since that time, vasculature-mediated damage
to tumors has been identified as an important constituent in
the tumor response to various forms of therapy, such as
hyperthermia, photodynamic therapy, cytokines, and some
drugs (7-13). In the last 5 years, an increasing amount of
attention has been focused on this possible new target, as
exemplified by the Gray Conference Proceedings published
in 1991 (14). Although these physical and chemical agents
have been shown to cause cessation of blood flow leading to
hemorrhagic or ischemic necrosis, there has been no
evidence for such an effect with ionizing radiation.

The recognition of the hallmarks of vasculature-mediated
injury depends on the time scale and pattern of injury that
can be seen shortly after the vessels have been damaged
(thrombosed, occluded by pressure, or ruptured). For
example, characteristic of vasculature-mediated injury is the
presence of patchy, large areas of necrosis, with individual
cords of cells surrounding the few vessels that are still
functional. Because of the variable latent period before
damage is expressed after radiation therapy, the absence of
this histology in tumors assessed shortly after irradiation
could not prove or disprove the relative contributions of
direct versus vasculature-mediated injury. Experiments in the
1960s had indicated that stromal damage might be expressed
in tumors only when the tumor mass was regrowing after
ineffective therapy (15). That study implied, although it did
not state, that the endothelial cells were not proliferating
during the regression phase and that the latent DNA damage
in them had not been expressed until regrowth occurred. The
injury to the cells, therefore, would not contribute much to
the extensive cell kill needed to cause local control, i.e.,
prevent local regrowth. It was, however, possible to show
that preirradiating the epidermis and dermis of mice before
implantation of tumor cells could slow down the tumor
growth rate by damaging vasculature, even though the tumor
cells themselves had received no treatment (16). This effect
became known as the ‘‘tumor bed effect.’’

The difficulty of resolving whether tumor vasculature has
any influence on local tumor control after radiotherapy has
been nicely addressed by a study reported in this issue of the
Journal by Budach et al. (17). Budach et al. have
ingeniously used as hosts for a series of different murine and
human tumors an inbred mutant mouse strain whose normal
tissue is approximately three times more sensitive to
radiation than that of other mouse strains. Their experimental
design ensured that the stromal component of the tumor
derived from the host, not from the original donor animals.
They have compared the growth characteristics of the tumors
in normal and radiosensitive hosts, both in untreated mice
and in mice with tumors regrowing after irradiation with x
rays. By giving appropriate ranges of single doses, they have
also established the x-ray dose needed to completely and
permanently eradicate irradiated tumors in 50% of the
animals (tumor control dose, TCD\textsubscript{50}). If direct damage to
tumor cells is the only factor determining the level of cell
depletion, we would expect the TCD\textsubscript{50} to be similar in the
radioresistant and normal mice. If, however, vasculature-
mediated injury is important after radiation therapy, we
would expect the TCD\textsubscript{50} to be lower in the mice that have a
threefold greater intrinsic stromal radiosensitivity. Indeed the
extent to which the TCD\textsubscript{50} is reduced in a radiosensitive
host could in theory be used to calculate what fraction of the
damage is direct versus indirect.

Budach et al. (17) have convincingly shown that the
intrinsic radiosensitivity of the host stroma has no influence
on the TCD\textsubscript{50}. They have used large, single x-ray doses and
made the tumors uniformly radioresistant during irradiation
by using clamps to block the blood flow to the tumor-
bearing leg. These features are both irrelevant for clinical
applications, but this experimental design removes any
doubts about differences in hypoxic fraction or in the repair
capabilities in the different hosts. As an important control
earlier work by Budach et al. (18) has shown that, if tumors
are induced by carcinogens to originate from the normal
tissues of these radiosensitive mice, they have an increase in
radioresistance similar to that seen in the normal tissues.

The conclusions drawn from the present study, i.e., that
vascular damage is not a significant factor in tumor response
to radiotherapy, are not in conflict with conclusions drawn
from other studies (7,15,19). They show that radiation
therapy is not in the same category of cytotoxic agents as
hyperthermia, photodynamic therapy, cytokines, or some
chemotherapeutic agents. Even if some damage is mediated
through radiation injury to endothelial cells, the data
obtained by Budach et al. (17) clearly demonstrate that such
damage is a minor component after x rays.

It is curious that the data of Budach et al. (17) also shed
light on whether the altered regrowth rate of irradiated
tumors is a measure of latent injury being expressed in the
vasculature as new angiogenic demands are made (15). The
extent of the treatment-induced slowing of tumor growth is
similar in the different host animals, indicating similar
vascular effects at equal x-ray doses regardless of stromal
radioresistance. It could, however, reflect a saturation of the
tumor bed effect because the doses are so high, as described
by Begg and Denekamp (20). It is perhaps worth noting that
with the agents that have been shown to cause vascular
damage in tumors, the effect seems to be abrupt and is not
accompanied by a delayed or latent tumor bed effect. Indeed
tumors regrowing after vasculature-mediated injury show
growth curves parallel to those seen in untreated tumors or
may even show accelerated growth (21).

The report by Budach et al. in this issue nicely illustrates
the benefits that can be derived from lateral thinking and
from the use of an ingenious model system. Budach et al.
also indicate (by their appropriate exclusion of one subset of
tumors) the care that must always be exercised in making
sure that the tumor model is appropriate to the question
being posed and that the data are not compromised by
artifacts of immune rejection (22-24).
References


Notes

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