Scanning Microscopy

Volume 6 | Number 4 Article 14

10-24-1992

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Scanning Microscopy, Vol. 6, No. 4, 1992 (Pages 1035-1040) 0891-7035/92$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

ULTRASTRUCTURAL EFFECTS OF THERAPEUTIC IRRADIATION ON HUMAN EPITHELIAL TUMORS

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(Received for publication May 7, 1992, and in revised form October 24, 1992)

Abstract

Irradiation induces several cellular changes leading to death of cancer cells and normal cells which is followed by repairing processes of normal cells. We have studied the effects of therapeutic irradiation on head and neck cancers. Tissue samples taken before and during the radical irradiation (50-80 Gy) of the squamous cell carcinomas of the head and neck region were examined by light and electron microscopy. Nuclear atypia was most pronounced cellular change during irradiation. The tumor invasion pattern remained unchanged but the number of mitoses decreased. Lymphocytic infiltration increased at the beginning of the therapy (from 10 to 30 Gy) but decreased at the end of radiotherapy. The amount of neutrophils and the keratinization pattern remained almost unchanged at the light microscopical level. However, by electron microscopy, intracellular filaments and desmosomes tended increase slightly especially in tumors responding more favorable to the treatment. The changes in nuclear morphology pointing in a more undifferentiated direction are considered to be due to cell damage rather than to a more aggressive behavior of the tumor cells. This is in agreement with the simultaneous decrease in mitoses, which might partly be due to radiation induced arrest of tumor cells to the G2 phase. These observed changes correspond to animal studies in the literature and might be responsible for the disappearance of tumors during irradiation.

Key Words: Radiotherapy, squamous cell cancer, ultrastructure.

General Effects of Irradiation in the Treatment of Cancers

Radiation therapy continues to play an important role in the treatment of cancer (18, 24, 25). Especially lymphomas and testicular seminomas are highly radiosensitive. In addition, in squamous cell carcinomas of the head and neck region, radiotherapy is widely used alone or combined with surgery. Despite this, most of our knowledge concerning the morphological effects of irradiation are based on experimental studies on animals. According to these studies, such cellular effects of irradiation as atypical fibroblasts, swollen endothelial cells, telangiectasia of thin-walled vessels, and bizarre nuclear sizes and staining, are unspecific effects that may occur in reactions to injury other than irradiation (1). The lack of information concerning the cellular effects of irradiation in human tumors derives from the fact that biopsies are rarely taken from acutely irradiated regions. There are only a few studies concerning the effects of irradiation on human tumors (2, 5, 6).

The acute effects of irradiation have mostly been studied after a single high dose of irradiation in *in vitro* or in animal models. The main interest have been focused on alimentary epithelium where mitotic activity in the proliferative portion ceases after an irradiation dose. Pyknosis, karyohexia and karyolysis occur progressively, leading to cell necrosis. Maximal changes are observed 6-8 hours after irradiation according to Fajardo (7). After a single dose of 10 Gy or less, rapid recovery occurs. With an increasing single dose over 15 Gy, permanent cellular changes lead to cell death. Delayed epithelial changes include atrophy, necrosis, ulceration, cellular atypia, and dysplasia, even neoplasia. Stromal changes with fibrosis, atypical fibroblasts, fibrous exudate, necrosis and lack or paucity of cellular inflammatory exudate are included to late stromal irradiation-induced effects. Also many types of vascular changes; endothelial damage, thrombosis, medial fibrosis etc. have been described (7, 9, 19, 20).

In therapeutic irradiation the total tumor dose, 50-70 Gy, has been divided into small fractions. Usually 2 Gy

Figure 1. An electron micrograph of epithelial cells with oval or round nuclei (arrow) before irradiation (**a**). The nuclei atypia (arrow) is pronounced after 24 Gy (**b**) in a sample from the same tumor as in Fig. 1a. Bar = 1 μm.

per fraction daily are delivered five times weekly. The fractionation is based on the four **R**'s: **R**epair of normal tissue, **R**eoxygenation of tumor tissue, **R**edistribution of tumor cells within the cell cycle, and **R**epopulation by surviving normal cells. These changes make cancer cells more radiosensitive and give time for normal cells to recover from the radiation damage between fractions. Cellular effects of X-ray irradiation are based on a direct effect on DNA or an indirect effect via ionization of intracellular water which then attack DNA. Thus, the basic mode of action of radiotherapy is that it kills the cells *in situ* and allows the body to remove them (25).

Ultrastructural Effects of Irradiation in Human Head and Neck Cancers

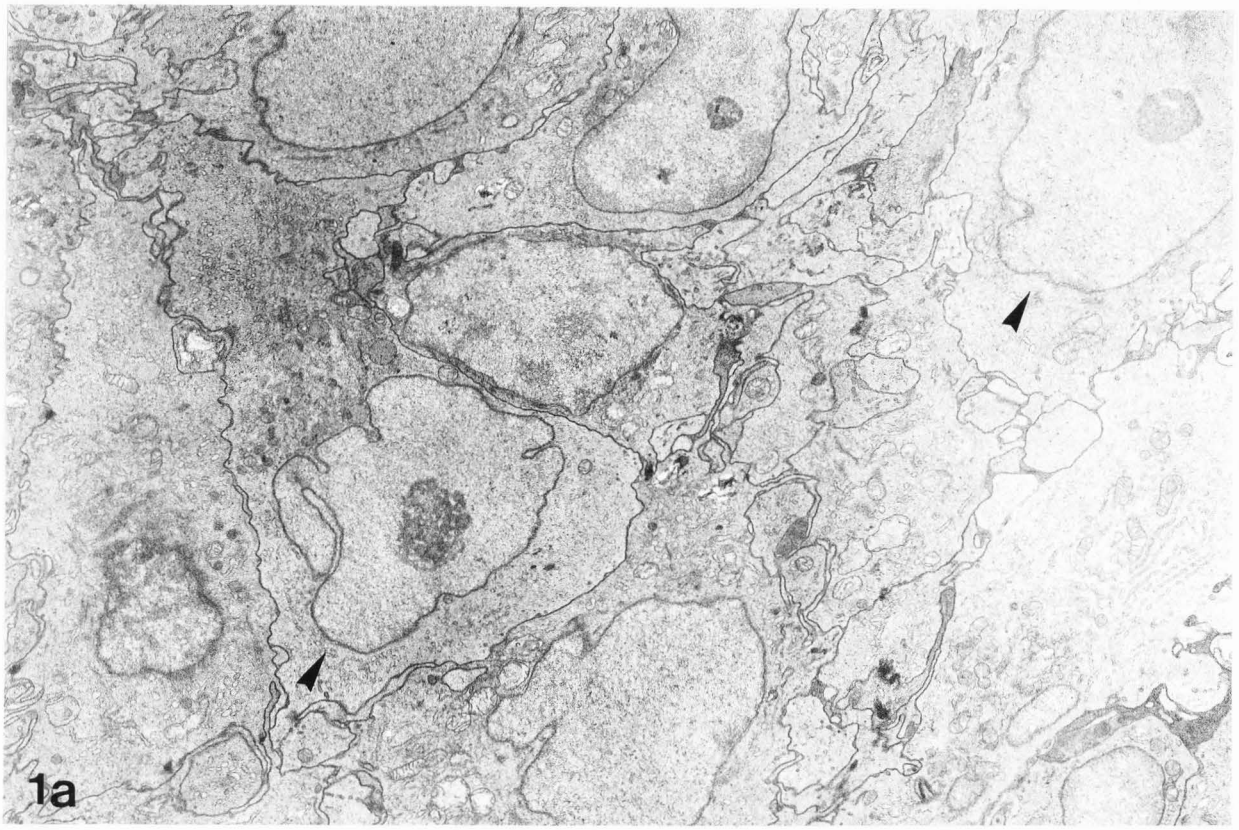
Since 1983, we have investigated the histopathological effects of well defined doses (2 Gy per fraction, five times weekly) of irradiation on the squamous cell carcinomas of the head and neck. Biopsies for light and electron microscopic studies have been taken before irradiation, after 10 to 30 Gy (one to three weeks of irradiation) and at the end of the therapy (if visible tumor left) from patients who received radical irradiation (over 50 Gy, mean 66 Gy) to their head and neck squamous cell carcinomas. The samples have been fixed and handled as earlier described (10)

In the first phase (10, 11, 12) multiple histologic parameters were identified and estimated on a scale of 1-4 from the samples obtained from the tumors. The parameters examined were keratinization, nuclear grade, mitosis, and the amount of fibrosis and inflammation. Briefly, the greater number on the estimation scale, the worse the situation was, e.g., in the degree of keratinization 1 denoted the presence of well defined extracellular pearls, 2 the presence of poorly formed extracellular keratin, 3 the formation of intracellular keratin, and 4 no identifiable keratin (4). At the ultrastructural level, the amount of intercellular filaments and desmosomes was also estimated on a scale from 1 to 4. In filaments 1 meant abundant filaments, 2 some filaments, 3 very sparse (some cells without and a few with), and 4 absence of filaments. An abundance of desmosomes was 1, some desmosomes 2, only a few and some cells without desmosomes was 3, and 4 was absence of desmosomes. In nuclear changes scale 1 meant that nuclear atypia was seen in less than 25% of cells, 2 that nuclear atypia was found in 25-50% of the cells, 3 in 50-75% of the cells, and 4 in over 75% of the cells, respectively. All the samples for light and electron microscopy were so coded that the microscopist did not know the time of biopsies in order to avoid the possibility of selection of study areas (10). In the second phase the above mentioned structural changes, especially keratinization pattern, were correlated to the treatment response of the tumors (13), and more samples and patients will be added to this analysis.

The most pronounced change caused by irradiation was the increase in nuclear grade (Fig. 1a, nuclei before irradiation and Fig. 1b, during irradiation from the same tumor). The mean of nuclear grade was 2.3 in pretherapy samples, 2.9 at 10 Gy, 3.3 at 30 Gy and 3.2 at the end of the treatment. The invasive pattern remained unchanged and the number of mitoses decreased in the irradiated tumor cells from 2.3 in pretherapy samples to 0.7 at 30 Gy. The number of lymphocytic inflammatory cells increased in the tumors with an irradiation dose of 10-30 Gy but with 60 Gy the number seemed to decrease. The amount of neutrophils in the tumors did not clearly depend on the irradiation dose. The mean keratinization levels of tumors at light microscopic level was not markedly altered. On the other hand, the keratinization pattern studied at the ultrastructural level showed that the intracellular filaments and the amount of desmosomes slightly increased in the tumors with irradiation dose of 10-30 Gy. The mean level of intracellular filaments in pretherapy samples was 2.8, 2.6 in 10 Gy and 2.0 in 30 Gy. The mean level of desmosomes was 2.6, 2.5, and 2, respectively. The amount of fibrosis in the tumor tissue was clearly correlated to the amount of irradiation of the tumor (Fig. 2).

In addition, in almost all irradiated specimens, there were tumor cells with a typically blown up nucleus. In the stromal fibroblasts, there appeared enlarged nuclei of differing size and shape. Their nuclei were also hyperchromatic (Fig. 2). These fibroblasts are comparable to the previously described atypical fibroblasts. These cells could also be identified in the electron microscopic specimens. For a detailed analysis of the scores during irradiation see our earlier works (13).

In the second phase (13) these changes were correlated to the outcome of the patients studied according to the general guidelines of response evaluation during and after cancer treatment (15). In the patients with complete response (N = 4), the amount of desmosomes and intercellular filaments increased in three patients and remained the same or decreased in one patient. In contrast, in patients with dissemination, they increased in



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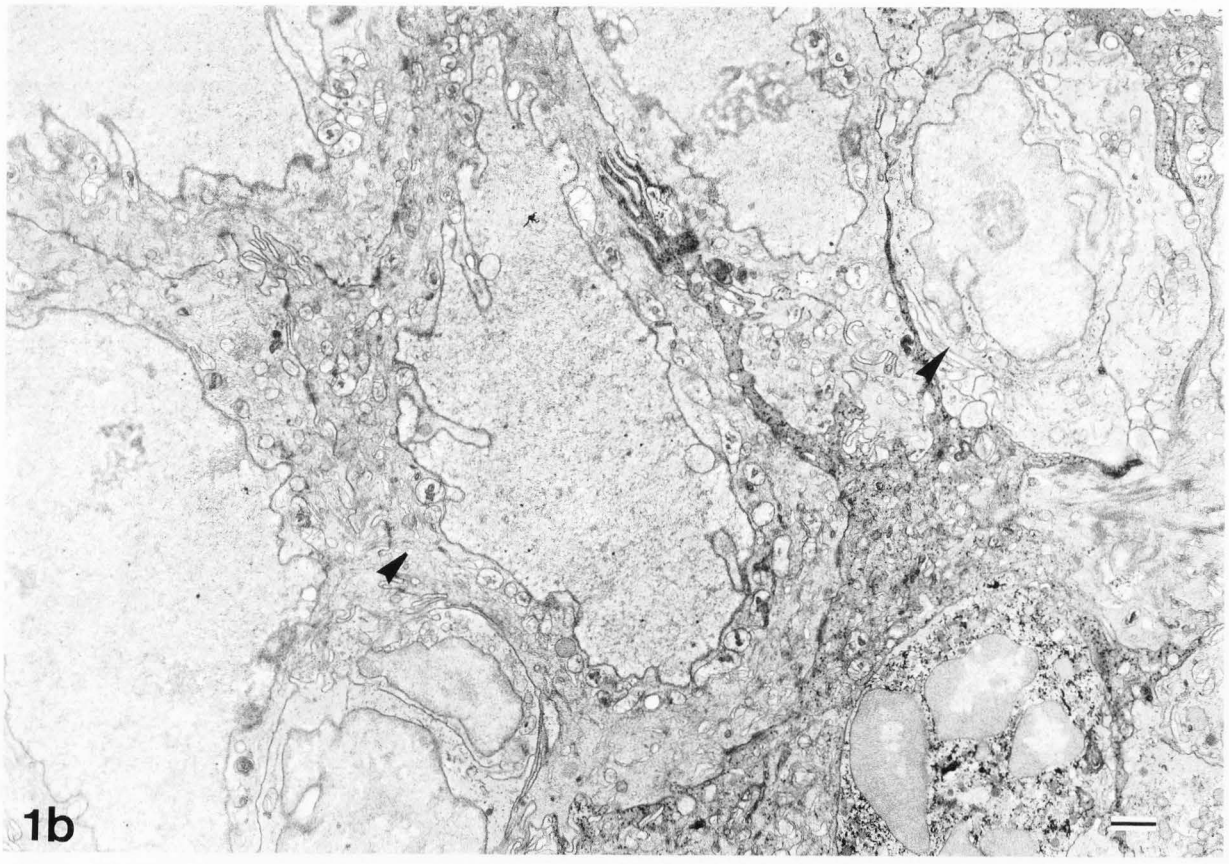

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Figure 2. An atypical fibroblast (arrow) and fibrotic stroma after irradiation. Bar = 1 μm.

none of the three cases. In locally persistent or recurrent tumors (N = 15), the amount of desmosomes increased in 40%, remained the same in 47% and decreased in 13% of the patients during the treatment. The corresponding figures for intermediary filaments were 20%, 33% and 47%.

Discussion and Conclusion

Radiotherapy continues to be of great importance in the treatment of malignancies. Therefore, studies concerning the effect of irradiation at the cellular level are important. Surprisingly few well controlled studies with well known irradiation doses and histologic tissue samples have been performed in humans. Most studies have been performed on experimental animals usually after single high dose irradiation whose biological effect might be totally different from that of fractionated irradiation although the total dose is the same.

Irradiation causes marked histologic changes in the cells. These include an increase in the nuclear atypia. These changes probably do not indicate a change in the tumor in a more malignant direction; the apparent nuclear atypia reflects degenerative changes in the tumor cell nucleus and the invasive change represents necrosis and cell death, which leads to disintegration of the invasive pattern. This conception is also supported by the finding that the number of mitoses in the irradiated tumors consistently decreased. This may be the result of the effect of irradiation on dividing cells. Cells in the G2 phase are more susceptible to irradiation and therefore, cells which are dividing more frequently are more likely to be affected by the irradiation, while less frequently dividing cell clones are more likely to survive.

Keratinization has been shown to occur during irradiation in those tumors of cervical carcinoma which respond better to irradiation (8). In that study, the changes in keratinization pattern at light-microscopic level were not seen in viable tumor parts of the samples, as had been noted in our preliminary immunohistochemical studies. On the other hand, when normal epithelium grows to the tumor region, it is keratinized squamous epithelium, and this might partly explain the findings of Glucksman (8). However, also in that study, intracellular filaments and desmosomes, which are part of the keratinization pattern, increased in some tumors. Desmosomes are associated with intercellular junctions, since one of their important functions is to ensure cell-to-cell adhesion and connection with tonofilaments in the cytoplasm, and their level has been shown to decrease in many epithelial tumors, more so in high grade tumors. It has also been shown, in a study of bladder carcinomas, that when no invasion occurred, the desmosome level remained similar to that in controls, but when invasion was present, their level decreased (14, 21, 22). The findings of these earlier studies support the hypothesis that the increase in the level of desmosomes during irradiation shown in our study might be responsible for the adhesion tumor cells to the irradiated region. This hypothesis is reinforced by the fact that in the locally persistent or recurrent tumors, the amount of desmosomes increased during irradiation in about half of the patients.

Fibrosis, containing atypical fibroblasts, are common response to connective tissue injury. It has been shown that fibroblasts isolated from irradiated breast tissue can produce oncofetal fibronectin and alfa-actin isoform which is specific to smooth muscle cells (3). These fetal myofibroblast-like cells were also observed in our studies. The contribution of these kinds of changes to possible later secondary malignancies or recurrences, is not yet known. On the other hand, pseudomalignant tissue reaction can occur following radiation, as shown by Weidner *et al.* (24). These kind of changes should not misinterpret as malignant (26). The increase in lymphocytic infiltration at the beginning of the treatment, might be partly due to the changes in host-tumor interaction, and lymphocytes might take care of cell debris. Toward the end of radiotherapy, tumor tissue was replaced by fibrosis and thus lymphocytic infiltration decreased.

It is not easy to predict the response of a tumor to radiotherapy (17, 22). There are no good diagnostic tools and tumor cell heterogeneity affects on the results and the sampling. Thus, more tumors and samples should be analyzed before final conclusion is drawn as to the possible predictive value of intracellular changes for the final outcome of the patients. In addition, the normal tissue reactions should be further evaluated during the irradiation and after it.

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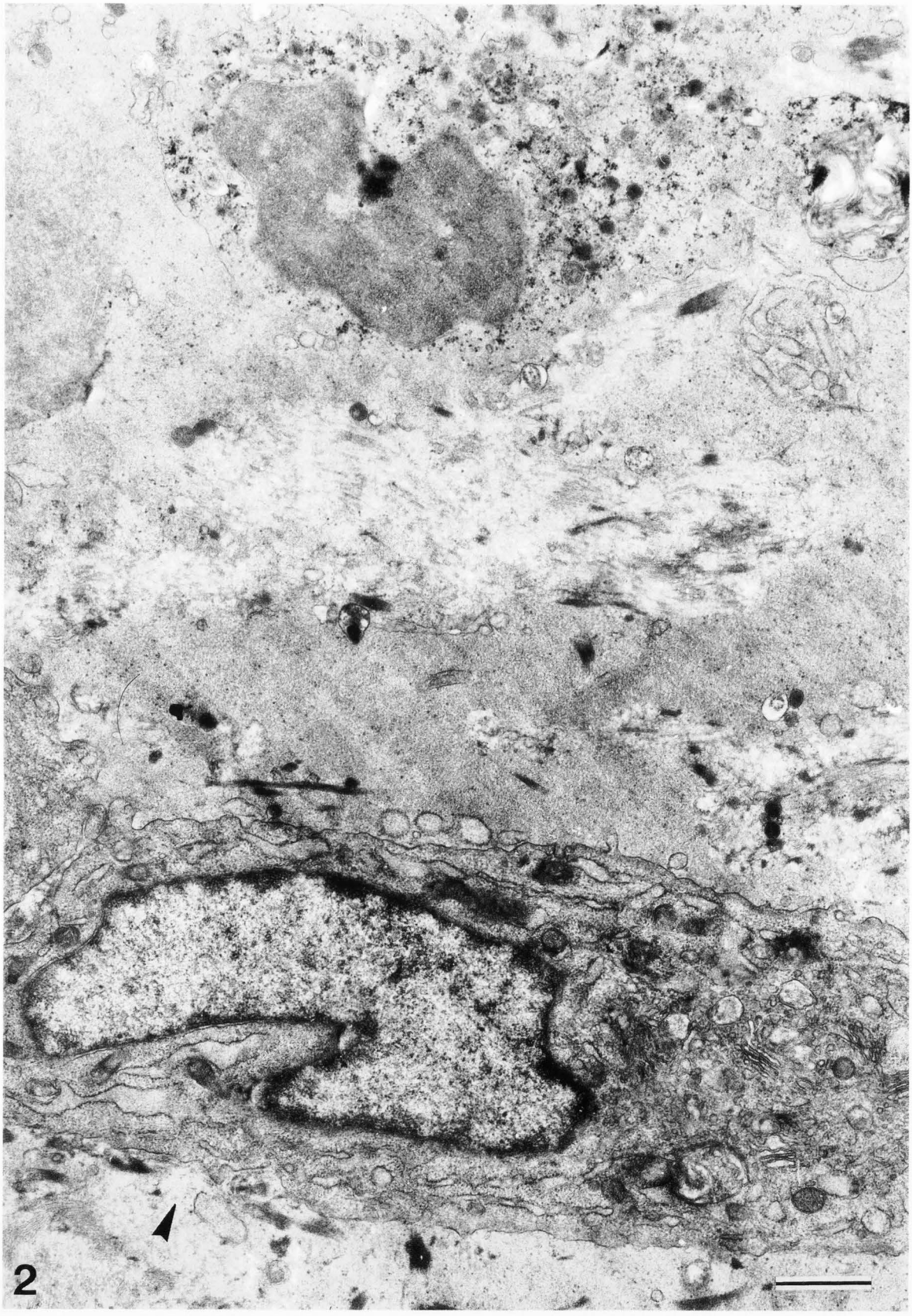
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Discussion with Reviewers

T.M. Seed: Do any of the single response endpoints noted, or any combination of endpoints, have prognostic potential in terms of "cure" or "state of remission" etc.
Author: This is an important, but a difficult question. In head and neck cancer treatment there are not yet any reliable prognostic tool for cure. The rapid macroscopical disappearance of tumor during irradiation does not correlate to the final cure (see e.g., text reference 16). Differentiation level does not correlate to the final result. Thus our aim has been to study, if such morphological features, like keratinization pattern, exist; but the number of patients is still too small to do final conclusions and we are collecting more patients and samples, and as you know this kind of clinical study continues slowly.