Significance of the Response of Quiescent Cell Populations within Solid Tumors in Cancer Therapy

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(Received on September 6, 2001)
(Revision Received on December 25, 2001)
(Accepted on January 22, 2002)

Quiescent cell/Micronucleus/Apoptosis/BrdU/DNA-damaging treatment

In analyzing the response of quiescent (Q) cells in solid tumors, we have developed a combined method with a micronucleus (MN) assay and the identification of proliferating (P) cells by 5-bromo-2’-deoxyuridine (BrdU) and an anti-BrdU monoclonal antibody. Using this method, the responses of Q tumor cells as well as total tumor (P+Q) cells within murine solid tumors to various DNA-damaging treatments were evaluated. Based on this evaluation, combining with tirapazamine, a well-known bioreductive agent, and/or heat treatment at mild temperatures was thought to be a promising modality for cancer therapy in terms of conventional anticancer treatment-resistant Q cell control. Recently, our method for detecting the Q-cell response using P cell labeling with BrdU and the MN frequency assay was also shown to be applicable to an apoptosis detection assay. Meanwhile, our method for detecting the intratumor Q-cell response was also applicable toward high linear energy transfer radiation, including reactor neutrons. Thus, using our method, a new neutron capture compound that has the potential to be distributed in neutron capture therapy-resistant intratumor Q cell populations is now under development.

INTRODUCTION

Human solid tumors are thought to contain moderately large fractions of quiescent (Q) tumor cells that are out of the cell cycle and stop cell division, but are viable compared with established experimental animal tumor lines that have been employed for various oncology studies1). The presence of Q cells is probably due, in part, to hypoxia and the depletion of nutrition in the tumor core, which is another consequence of poor vascular supply1). As a result, Q cells are viable and clonogenic, but stop cell division2). In general, radiation and many DNA-damaging chemotherapeutic agents kill proliferating (P) tumor cells more efficiently than Q tumor cells, resulting in many clonogenic Q cells remaining following radiotherapy or chemotherapy1). Therefore, it is thought to be harder to control Q tumor cells than to control P tumor cells, and many post-radiotherapy recurrent tumors are thought to result partly from the regrowth of Q tumor cell populations that could not be sufficiently killed by radiotherapy2). Further, sufficient doses of drugs cannot be distributed within Q tumor cell populations mainly due to heterogeneous and poor vascular distributions within solid tumors3,4).
Thus, one of the major causes of post-chemotherapy recurrent tumors is thought to be an insufficient dose distribution in the Q cell fractions\(^3,4\). Our newly developed method for selectively detecting the response of intratumor Q cells\(^5\) has made it possible to evaluate the usefulness of various modalities for cancer therapy in terms of the effectiveness on the intratumor Q cell populations. Based on the characteristics of the intratumor Q-cell responses to various DNA-damaging treatments, more effective and useful modalities for cancer therapy can be developed.

PREVIOUS CONSENSUS CONCERNING QUIESCENT CELLS IN SOLID TUMORS

Q cells are operationally defined as those cells not in active proliferation during the course of the time during which measurements are obtained\(^6\). We consider the term “quiescent” to include all tumor cells out of the cycle, irrespective of the reason. The Go state, in contrast, is confined to viable cells that are out of the cycle under normal physiological conditions (i.e. not due to nutrient deprivation in itself), and that can be recruited into active proliferation by an appropriate stimulus\(^7\). The best examples of these cells are found in normal intact tissues (liver, salivary gland etc.).

Studies in vitro using plateau phase cultured tumor cells\(^6,8,9,10\) have revealed the following: 1) Q cells have G1 DNA contents; 2) Q cells have approximately 50% cellular RNA compared with P cells; 3) the volume of Q cells was approximately half that of P cells; 4) the viability was > 95% regardless of the proliferative state; 5) the colony-forming ability decreased as the cells entered the quiescent state; 6) the length of the Q to P transition varies and the depth of the Q state depends on the amount of time the cells have been quiescent; and 7) Q cells are more sensitive to ionizing radiation than P cells; and 4) Q cells showed greater repair of potentially lethal damage (PLDR) than P cells. Using fluorescence-activated cell sorting based on the diffusion properties of the fluorochrome Hoechst 33342, another study\(^14\) suggested that hypoxic regions of KHT tumors contain a smaller percentage of actively proliferating cells.

Consequently, the following is now thought to be the characteristics of Q cells in solid tumors: 1) Q cells have G1 DNA contents; 2) Q cells are more sensitive to radiation and have larger PLDR capacities than P cells; 3) the Q cell populations have a large hypoxic fraction mainly due to the limitation of oxygen diffusion; and 4) Q cells have the potential to be recruited into the P state by appropriate stimuli, such as radiation\(^15\).

However, the response of intratumor Q cells in vivo had not been directly detected until we developed the method described in the following section.

ESTABLISHMENT OF OUR METHOD FOR DETECTING THE RESPONSE OF QUIESCENT CELLS IN SOLID TUMORS TO DNA-DAMAGING TREATMENT

The preliminary study described in the following section was performed beforehand\(^16\). To determine the radiosensitivity of non-S phase tumor cells in vitro, survival curves of SCC VII tumor cells were obtained after a short block with S phase cell toxin hydroxyurea. Dose-response curves of micronucleus (MN) frequency in non-S phase cells following pulselabeling with BrdU were also determined by excluding S phase cells with immunofluorescence staining for 5-bromo-2’-deoxyuridine (BrdU) using a monoclonal anti-BrdU antibody and a fluorescein isothiocyanate (FITC)-conjugated antimouse IgG antibody. Both the dose-response curves of the MN frequency and the cell survival curves were analyzed by a linear quadratic formula (Surviving fraction =}
exp(−αD − βD^2), MN frequency = aD + bD^2 + c). A good correlation was observed between the $\alpha/\beta$ and a/b ratios. In both BrdU-unincorporated (= non-S phase) and asynchronous cell cultures, the regression lines between the surviving fraction (SF) and the MN frequency were statistically identical (Fig. 1). Therefore, it was shown that cell-survival curves, which cannot be obtained directly by the routine colony formation technique, can be calculated using the MN frequency and the regression line between the SF and MN frequency for asynchronous cell cultures. Therefore, it was thought to be possible to detect the response of Q cells in solid tumors using immunofluorescence staining for BrdU and the MN frequency assay following continuous intratumor P cell labeling with BrdU.

A subsequent in vivo study was conducted to establish our method for detecting the response of intratumor Q cells to X-ray irradiation\(^5\). SCC VII tumor-bearing C3H/He mice were irradiated after being given 10 injections of BrdU at an interval of 12 hours to label all P cells in solid tumors. The tumors were then excised and trypsinized. The thus obtained tumor cell suspensions were incubated with cytochalasin-B (which blocks cytokinesis) for 48 to 72 hours, and the MN frequency in these cells without BrdU labeling was determined using immunofluorescence staining for BrdU. This MN frequency was then used to determine the SF of the BrdU-unlabeled cells from the regression line obtained between the MN frequency and the SF determined for the total cells in the tumor. Thus, a cell-survival curve could be determined for cells not labeled by BrdU, which could be regarded for all practical purposes as the Q cells in a solid tumor (Fig. 2). Assays performed both immediately and 24 hours after irradiation of normally aerated tumors showed that the BrdU-unlabeled cells were more radioresistant and had a higher PLDR capacity than the tumor cells as a whole. Moreover, when the assay was performed immediately after irradiation of both normally aerated and totally hypoxic tumors, BrdU-unlabeled cells were found to contain a much larger hypoxic fraction (HF) than the tumor cells as a whole (Fig. 3).

Needless to say, the induction of a MN requires division of the cell nucleus\(^5,16\). The duration of incubation with cytochalasin-B allowed Q cells to be recruited into the cell cycle. Thus, the optimal incubation period was determined for each DNA-damaging treatment so that the maximum rate of binuclear tumor cells could be observed. During continuous labeling with BrdU for over 5 days, the shift of cells from P to Q population could result in labeled Q cells. These cells were excluded when we scored micronuclei in binuclear cells showing only red fluorescence by propidium iodide (PI) for DNA staining, because these cells were stained with FITC. In addition, in some differentiating human cancers, Q tumor

![Fig. 1. Correlation between the normalized MN frequency (MN frequency - C, where C is the MN frequency in cells from tumors in animals not given radiation) and the surviving fraction. The closed and open circles represent asynchronous and BrdU-unincorporated tumor cell cultures, respectively. The bars represent the 95% confidence limits. The number of each point was 9 to 12. The regression lines between the surviving fraction and the normalized MN frequency for asynchronous and BrdU-unincorporated tumor cell cultures were statistically identical.](https://academic.oup.com/jrr/article-abstract/43/1/11/910211)
cells may predominantly be the non-proliferating end cells of a hierarchically structured population. However, rapidly growing transplantable rodent tumors are now thought to well reflect the nature of many rapidly growing human tumors. Thus, our method appeared to be a very useful way for detecting the responses of Q cells in solid tumors to various treatments, and was established as a promising system to determine intratumor Q-cell responses to a DNA-damaging treatment.

**DIRECTLY DEMONSTRATED CHARACTERISTICS OF QUIESCENT CELLS IN MURINE SOLID TUMORS USING OUR METHOD**

Using our method after low linear energy transfer (LET) radiation exposure to tumor-bearing mice, as stated above, the following characteristics of Q cells in murine solid tumors were clarified: 1) Q tumor cells are more radioresistant than total (P + Q) tumor cells; 2) Q cells have larger PLDR capacities than total cells; and 3) Q-cell populations include a larger size of HF than total cells. Using the HF values and the ratios of P cells to total cells (= growth fraction) in two different mouse tumors, the ratios of the clonogenicity of Q cells to that of P cells were
calculated. Both of the ratios were significantly less than 1.00, indicating that the clonogenicity of Q cells is lower than that of P cells. It has been reported that solid tumors contain hypoxic cells due to the limitations of oxygen diffusion (chronic hypoxia) and the temporary occlusion of vessels or the slowing of blood flow (limitations of perfusion or acute hypoxia). Thus, the administration of nicotinamide before irradiation or the placement of mice in a circulating carbogen (95% O2/5% CO2) chamber for 30 minutes before and during irradiation altered the acutely and chronically HF of the P- and Q-cell populations in a manner that was dependent on the tumor system. These treatments indicated that the HF of Q cells is largely comprised of chronically HF with a smaller proportion of the acutely HF. Further, fractionated X-ray irradiation and continuous BrdU labeling until the time of the second irradiation indicated recruitment from the Q to P state during fractionated irradiation. In addition, in SCC VII squamous cell carcinoma tumors, reoxygenation after initial γ-ray irradiation to tumor-bearing mice was completed more slowly in chronic hypoxia-rich Q tumor cell fractions than in acute hypoxia-rich total tumor cell populations.

In response to the administration of chemotherapeutic agents, the chemosensitivity of Q tumor cells to cisplatin was lower than that of the total tumor cell population. However, when cisplatin was used, the sensitivity difference between the Q cells and the total cells was greater than that for X-ray irradiation. This difference can probably be attributable to the uneven distribution of cisplatin in the Q cells due to the heterogeneity of tumor vasculature, compared with the homogeneous delivery of X-rays throughout solid tumors. Following intraperitoneal cisplatin administration to tumor-bearing mice, the Q cells showed larger PLDR capacities than the total cells, and the PLDR pattern of each cell population was similar to that after γ-ray irradiation.

Meanwhile, the Q-cell sensitivity to tirapazamine (TPZ, SR-4233, WIN 59075, 3-amino-1,2,4-benzotriazine-1,4-dioxide), a well-known bioreductive agent that preferentially kills hypoxic cells, was greater than that of the total tumor cell population. Q cells showed greater PLDR capacities following a TPZ treatment than the total cells. However, TPZ caused a much smaller PLDR capacity in both the total and Q cells. This was compatible with Brown’s previous report showing that DNA breaks induced by TPZ are repaired much less readily than those produced by X-irradiation, because active TPZ radicals are produced at high local concentrations by activating enzymes close to the DNA. In addition, two fractionated TPZ administrations and the determination of the P cell ratios in the tumors at the time of the second administration indicated that the use of TPZ in the treatment of rodent solid tumors causes a shift from the P to Q state in vivo.

With regard to the oxygenation
status in SCC VII solid tumors after drug administration. TPZ and cisplatin reduced the HF after a treatment, especially in Q cells; this tendency was particularly marked with TPZ. In contrast, bleomycin increased the HF after treatment. Both reoxygenation following γ-ray irradiation or a bleomycin treatment and a subsequent return to the pretreatment levels of HF following a TPZ or cisplatin treatment (rehypoxiation) occurred more rapidly in acute hypoxia-rich total cells than in chronic hypoxia-rich Q cells (Fig. 4).

On the other hand, a heat treatment has been demonstrated to be effective as an adjuvant modality to radiotherapy. Laboratory experiments showed that heating for 30 to 60 min at relatively high temperatures, i.e. > 43 to 44°C, damages intratumor blood vessels and kills tumor cells. In addition, hyperthermia causes direct cellular radiosensitization. However, currently available hyperthermia devices have been ineffective in raising the temperature of human tumors sufficiently to cause vascular damage, kill tumor cells, and directly radiosensitize the tumor cells. Therefore, it was suggested that hyperthermia might improve tumor oxygenation and thus indirectly radiosensitize tumors through an increase in the tumor blood flow in previous clinical studies in which a heat treatment was shown to improve the effectiveness of radiotherapy. According to our reports, mild temperature hyperthermia (MTH) without direct cytotoxicity or direct radiosensitization effects (40.0°C, 30–60 min) before irradiation decreased the HF in SCC VII tumors, even when combined with the administration of an acute hypoxia-releasing agent, nicotinamide. In contrast, MTH did not decrease the HF when the tumor-bearing mice were placed in a circulating carbogen (95% O2/5% CO2) chamber, where chronic hypoxia is released. Therefore, MTH was thought to preferentially oxygenate the chronically HF. In addition, MTH decreased the HF of chronic hypoxia-rich Q cells of SCC VII tumors more markedly than the acute hypoxia-rich total cell population, and the minimum values of HFs of both total- and Q-cell populations were obtained 6 hr after MTH. Two days after MTH, the HF of the total tumor cells returned to almost that of unheated tumors. In contrast, the HF of...
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Q cells did not return to the level of unheated tumors until 1 week after MTH \(^{36}\) (Fig. 5). These findings also supported that MTH could preferentially oxygenate chronic hypoxia-rich Q cell fractions in the tumors. Further, the time courses of changes in the decrease in the HFs of total-and Q-cell populations after MTH suggested that irradiation within 12 hours after MTH might be a potentially promising therapeutic method for controlling radioresistant Q tumor cells, especially when it is difficult to elevate the tumor temperature high enough to cause vascular damage, kill tumor cells, and directly radiosensitize the tumor cells within solid tumors.

CHARACTERISTICS OF INTRATUMOR QUIESCENT CELLS IN NEUTRON CAPTURE THERAPY

A neutron capture reaction in boron \(^{10}\text{B}(n,\alpha)^7\text{Li}\) is, in principle, very effective for destroying tumors, provided that a sufficient amount of \(^{10}\text{B}\) can be accumulated in the target tissue and a sufficient number of very low energy thermal neutrons can be delivered to the target. The two particles generated in this event carry a high LET and an average total kinetic energy of 2.34 MeV, and have a short range of approximately one cell diameter, resulting in the induction of a high relative biological effectiveness (RBE) \(^{37}\) . Gadolinium neutron capture reaction \(^{157}\text{Gd}(n,\gamma\text{e}^{-}\text{e}^{-})^{158}\text{Gd}\) results in the emission of \(\gamma\)-rays with a maximum energy of 7.9 MeV, followed by a series of secondary \(\gamma\)-rays and 29 to 180 keV internal conversion electrons \(^{38}\) . On such gadolinium neutron capture therapy (NCT), the internal conversion electrons and Auger electrons are thought to play important roles in microscopic energy deposition; these electrons are responsible for about 15% of the total absorbed dose in gadolinium-treated tumors \(^{39}\) .

In clinical trials of NCT for human malignant brain tumors and malignant melanomas, \(p\)-boronophenylalanine-\(^{10}\text{B}\) (BPA) and sodium borocaptate-\(^{10}\text{B}\) (BSH) have been employed as \(^{10}\text{B}\)-carriers \(^{37}\) . Also, in our animal studies on intatumor Q-cell responses to a neutron capture reaction, BPA and BSH were mainly used as neutron capture \(^{10}\text{B}\)-compounds. \(^{18}\) Without \(^{10}\text{B}\)-compounds, although Q cells showed a lower sensitivity than the total cells, neutron irradiation reduced the difference in the \(\gamma\)-ray sensitivity between the total and Q cells. With \(^{10}\text{B}\)-compounds, even when the \(^{10}\text{B}\) concentrations within solid tumors at the time of reactor neutron irradiation were similar to each other, BPA increased the sensitivity of the total cells to a greater extent than BSH. However, the sensitivity of Q cells treated with BPA was lower than that in BSH-treated Q cells. \(^{19}\) The difference in the sensitivity between the total and Q cells was greater with \(^{10}\text{B}\)-compounds, especially
with BPA\textsuperscript{40,41} (Fig. 6). Q cells showed greater PLDR capacities than the total cells. γ-Ray irradiation and neutron irradiation with BPA induced larger PLDR capacities in each cell population. In contrast, thermal neutron irradiation without the $^{10}$B-compound induced the smallest PLDR capacity in both cell populations.\textsuperscript{20} The use of the $^{10}$B-compound, especially BPA, resulted in an increase in the PLDR capacity in both cell populations, and made the PLDR patterns of the two cell populations look like those induced by γ-ray irradiation\textsuperscript{42}. In both the total and Q tumor cells, the HFs immediately after neutron irradiation increased suddenly.\textsuperscript{21} Reoxygenation after each neutron irradiation occurred more rapidly in the total cells than in the Q cells.\textsuperscript{22} In both cell populations, reoxygenation appeared to be rapidly induced in the following order: neutron irradiation without $^{10}$B-compounds > neutron irradiation following BSH injection > neutron irradiation following BPA administration > γ-ray irradiation\textsuperscript{43}. Further, γ-ray irradiation following initial thermal neutron irradiation and continuous BrdU labeling until the time of the second irradiation indicated that\textsuperscript{23} the use of the $^{10}$B-compound, especially BPA, in thermal neutron irradiation causes recruitment from the Q to P population\textsuperscript{44}. These findings suggested that thermal neutron irradiation without $^{10}$B-compound reduces the difference in the sensitivity between the total and Q cells, and that the $^{10}$B from BPA is more dependent on the drug-uptake potential of tumor cells than that from BSH, because P cells are thought to have a greater potential for drug uptake due to their aerobic and well-nourished condition, mainly due to their rich and homogeneous vascular distribution\textsuperscript{3,4}.

With regard to gadolinium NCT,\textsuperscript{24} water-soluble non-enriched gadolinium-containing contrast media for magnetic resonance imaging (MRI) was not suitable as a neutron capture compound in NCT, because non-enriched gadolinium in the form of a clinically used non-ionized contrast agent for MRI was too water-soluble to be accumulated in tumor tissue for a sufficient period during neutron irradiation\textsuperscript{45}. Recently, for the accumulation of gadolinium at high concentrations in tumor tissue, microcapsules containing gadolinium for intra-arterial injection have been designed and synthesized\textsuperscript{46}.

**Fig. 6.** Dose-response curves of the normalized MN frequency (MN frequency - C, where C is the MN frequency in cells from tumors in animals not given radiation) as a function of the radiation dose for the total (solid symbols) and Q (open symbols) tumor cell populations. Reactor thermal neutron beams were exposed to SCC VII tumors after the administration of no neutron capture drug (circles), BSH (125 mg/kg, i.p., 20 min later) (squares) or BPA (1,500 mg/kg, p.o., 3 hr later) (triangles). As a control irradiation, other tumors were irradiated with γ-rays alone (open squares with a diagonal). Only mean values are shown, to avoid confusion.

**ATTEMPTS TO ENHANCE THE RESPONSE OF INTRATUMOR QUIESCENT CELLS IN THE TREATMENT OF SOLID TUMORS**

Solid tumors, especially human tumors, are thought to contain a high proportion of Q cells\textsuperscript{1}. In the solid tumor core, hypoxia and the depletion of
nutrition partly due to poor vascular supply are thought to cause a quiescent status of tumor cells\(^1,3,4\). It has been reported that Q cells have lower sensitivity to irradiation and anticancer drugs than P cells in solid tumors \textit{in vivo}\(^1,5,21,25\). This means that more Q cells can survive after an anticancer treatment than P cells. Consequently, the control of Q cells has a marked impact on the outcome of anticancer therapy. Based on the above characteristics of Q cells within solid tumors, the following methods for enhancing the response of intratumor Q cells were suggested.

\textbf{a) Increasing the sensitivity of Q cells:}

When the solid tumors were irradiated with high LET radiation fast neutrons or reactor neutrons, the difference in intrinsic radiosensitivity between total tumor and Q cells was markedly reduced, compared with low LET X-rays or \(\gamma\)-rays, especially when large radiation doses were applied\(^{45,47,48,49}\) (Fig. 7). TPZ administration and/or MTH combined with irradiation and/or chemotherapeutic agent treatment were also effective for enhancing intratumor Q-cell sensitivity\(^{50,51,52}\) (Fig. 8). The interaction of TPZ with irradiation was shown to be essentially additive \textit{in vitro}\(^53\). In addition, although MTH increased the effects of TPZ on the total and Q cells in solid tumors, the effect was more marked in Q cells\(^\text{28}\). Further, combined nicotinamide administration and carbogen inhalation also elevated the sensitivity of the total and Q cells to some degree\(^\text{54}\).

\textbf{b) Inhibition of PLDR from DNA damages:}

PLDR by intratumor Q cells was inhibited more strongly with the high LET radiation of fast neutrons than with low LET radiation X-rays, especially when large doses were delivered\(^{47,48}\) (Fig. 7). PLDR of Q cells after the low LET radiation X-ray or \(\gamma\) ray irradiation was efficiently inhibited by the hypoxic cell sensitizer misonidazole or TPZ administration immediately after irradiation\(^{55,56,57}\). Especially, the post-irradiation administration of TPZ exhibited not only a PLDR-inhibiting effect, but also a large post-irradiation radiosensitizing effect on both the total and Q tumor cell populations \textit{in vivo}\(^57\).

\textbf{c) Exploiting a large HF in Q cells:}

Because Q cells have a large HF, especially a large chronically HF, compared with the total tumor cells, the use of hypoxia-selective cytotoxin TPZ and/or chronic hypoxia-releasing MTH combined with radiation and/or chemotherapy was thought to be promising\(^\text{58}\). These treatments were very effective in terms of enhancing the Q-cell response in radiotherapy or chemotherapy\(^{50,51,52}\) (Fig. 8), including NCT\(^\text{41,59}\). Further, a combined treatment with TPZ and MTH, regardless of whether other cytotoxic treatments, such as \(\gamma\)-ray irradiation or chemotherapy, were included, was useful for sensitizing tumor cells \textit{in vivo} including Q cells even after an anti-angiogenic agent (TNP-470) treatment\(^\text{60}\). Recently, it was...
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also reported that combining with TPZ is still needed to enhance the Q-cell response on chemoradiation using the mitotic spindle poison paclitaxel, because no radiosensitization effect on combination with paclitaxel was induced in Q tumor cells61).

d) Exploiting recruitment after DNA-damaging treatment:

X-ray irradiation or thermal neutron irradiation combined with $^{10}$B-compound, especially BPA, caused recruitment from the Q to P state22,44). Thus, the fractionation modality may be effective in terms of Q-cell control. In contrast, a TPZ treatment alone caused a shift from the P to Q state in vivo30). Therefore, fractionated treatments with TPZ alone are thought to be ineffective for enhancing the Q-cell response. More studies on the relationship between fractionation techniques and the effects on intratumor Q cell are still needed.

e) Exploiting the characteristics of drug distribution:

Especially in NCT, some neutron capture compound, whether $^{10}$B-carrier, $^{157}$Gd-carrier, or other compounds, has to be combined with reactor neutron irradiation to control solid tumors as a whole37). The distribution of $^{10}$B from BPA in tumor cells is more dependent on the $^{10}$B uptake ability of the tumor cells than that from BSH. Meanwhile, the distribution of $^{10}$B from BSH is more dependent on the diffusion of the drug than that from BPA41). Consequently, BPA with reactor neutrons is effective for controlling the total cells (= P + Q cells), and the use of BSH combined with reactor neutron irradiation is thought to be rather effective to control radioresistant Q cells because of their low uptake potential. From the viewpoint of tumor curability as a whole, the combination of BSH and BPA may be useful in NCT45,49).
PERSPECTIVES

To date, we have employed the MN assay for selectively detecting the Q-cell response. Micronuclei have been reported to appear in dividing cells with chromosomal aberrations after a DNA-damaging treatment. The frequency of their appearance was reported to correlate with the degree of the treatment intensity and with the cell killing\(^\text{62,63}\). It has been demonstrated that some cells die through apoptosis after a treatment, and that apoptosis may be related to the tumor sensitivity to the treatment\(^\text{64}\). However, the contribution of apoptosis to determining the responses of tumors, as well as the induction of MN after DNA-damaging treatment\(^\text{65}\), depends on the cell type\(^\text{64,66}\). That is, the ratio of apoptosis or MN formation to the total cell death depends on the cell type\(^\text{64,66}\). Recently, in our research, whether based on the MN frequency or the apoptosis frequency, similar results concerning the radiobiological findings of intratumor total and Q cells were obtained\(^\text{67,68}\). Optimal hours after DNA-damaging treatment, solid tumors were excised, minced and trypsinized. The tumor cell suspensions thus obtained were fixed and the apoptosis frequency in cells without BrdU labeling was morphologically determined using immunofluorescence staining for BrdU (Fig. 2). In advance, the optimal time for tumor excision after each DNA-damaging treatment was determined so that the maximum value of apoptosis frequency could be histologically observed. Therefore, it was revealed that the apoptosis frequency, as well as the MN frequency, can be applied to our method for measuring the Q-cell response to DNA-damaging treatment, such as radiation within solid tumors in which the ratio of apoptosis to the total cell death is relatively high.

Using our method combined with the MN assay or apoptosis detection assay and identification of P cells by BrdU and anti-BrdU monoclonal antibody, we evaluated the radiosensitization effect on solid tumors upon a combination treatment with paclitaxel including the effect on intratumor Q cells\(^\text{61}\). Recently, it has been shown that the p53 tumor suppressor gene serves a critical role in maintaining the genomic stability during the cell-cycle checkpoints in the G1 and G2/M transition\(^\text{69,70,71}\), as an effector of DNA repair\(^\text{72,73}\) and apoptosis\(^\text{74,75}\). These actions of p53 are potentially critical in determining the effectiveness of ionizing radiation and/or chemotherapeutic agents. In the future, we plan to examine the relationship between the radiobiological characteristics of tumor cells, i.e., the total cells and Q cells, in solid tumors and the p53 status of the cells constituting the solid tumors, using tumor cell lines with identical genetic backgrounds except for p53 status.

Recent studies at other institutes\(^\text{76,77}\) have shown that the expression of sigma-2 (σ2) receptors, which represent a class of proteins originally classified as a subtype of the opiate receptors, may serve as a potential biomarker of cell proliferation of an entire tumor both before and after a treatment not only \textit{in vitro}, but also \textit{in vivo}, overcoming technical problems concerning biopsy specimens from solid tumors. We also plan to investigate the relationship between the expression of gene product and proliferation status \textit{in vivo}.

CONCLUSIONS

Based on the characteristics of Q cells in solid tumors directly clarified with our method for determining the Q-cell response, a combined treatment with TPZ and MTH is now thought to be very promising for conventional radiotherapy and/or chemotherapy from the viewpoint of not only Q-cell control, but also the entire tumor curability. Further studies are needed regarding the fractionation modality related to the Q-cell response for clinical applicability. For an NCT study, a new neutron capture compound having a high tumor specificity with a homogeneous distribution capacity throughout tumors, long retention in tumors and complete clearance from blood and normal tissues has to be developed. A \(^{10}\text{B}\)-containing \(\alpha\)-amino alcohol of BPA, \(L-\text{p-}
boronophenylalaninol-\(^{10}\text{B}\), with higher water solubility than BPA was designed and synthesized as a new
10B-carrier to develop biologically more selective BPA congeners for NCT\(^{78,79}\). Its usefulness in NCT has now been evaluated from various viewpoints, including the killing effect on Q tumor cells in comparison with the total tumor cell population using our method for detecting the intratumor Q-cell response\(^{60}\).

**ACKNOWLEDGEMENT**

This study was supported, in part, by Grants-in-Aid for Scientific Research on Priority Areas and Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and Japan Society for the Promotion of Science. First author is very grateful to Dr. Koichi Ando (Head, Clinical Radiation Biology and International Space Radiation Laboratory, National Institute of Radiological Sciences, Editor-in-Chief, Journal of Radiation Research) for the kind invitation to write this review article.

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