Hypoxic Cell Population in Human Tumor and its Implication to Radiotherapy

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ABSTRACT

Multi-target response law in cellular radiation biology has been extended to clinical dose fractionation schedules. Hypoxic cell component in tumors makes this extension very confused. Hall promoted rethinking of the oxygen effect in the radiotherapy. This paper presents this rethinking in terms of TCD9O or 90% tumor control dose.

INTRODUCTION

Oxygen dependence of cellular radiosensitivity has been well established for the ionizing radiation, i.e., x- or gamma-rays. Malignant tumors are believed to be composed of two different kinds of cell populations, i.e., aerobic or radiosensitive and hypoxic or less sensitive tumor cells. The experimental animal tumors have been reported to contain large foci of hypoxic cells. Dose-survival curve of multi-component cell population such as tumor was obtained by Powers and Tolmach.1) Their data suggest that sterilization of a tumor or tumor control dose (TCD) largely depends on the sensitivity of hypoxic cells and the percentage of hypoxic cell population in the tumor.

The idea that tumor contains large foci of hypoxic cell population was extended to human cancer radiotherapy. Much attention has been focused on hypoxic cells in radiobiology, especially in the tumor radiobiology. Very recently Hall2) suggested that oxygen effect is unimportant in clinical radiotherapy and promoted rethinking of the oxygen effect in human radiotherapy. His idea based on experi-
mental data reported by Suit et al. as follows:

Hypoxic cells may present in a tumor. However, throughout a course of radiotherapy (say, given in 30 fractions), such cells might be reoxygenized. Therefore, originally (i.e., at the beginning of radiotherapy) hypoxic cells may not remain oxygen-deficient throughout the 30 fractions of radiotherapy.

In this paper, additional rethinking will be presented whether Hall's idea is barking up the wrong radiotherapeutic tree or not.

DOSE-FRACTIONATION SCHEMA AND TCDp

To sterilize a human tumor, 6,000-7,000 rads are given in 30 fractions in six weeks, or 2,500-3,000 rads might be delivered in rare cases as a single fraction. Let us assume that human cancer cell lines have a similar sensitivity as animal tumors or "in vitro" cell lines, and have D0 (dose required to reduce survival fraction of 1/e in the straight portion of multi-target survival curve) of 100-160 rads in aerobic cells and of 300-400 rads in hypoxic cell population with an extrapolation number or target number (m) of two or three for both cell populations.

If each cell reacts independently to the ionizing radiation, TCDp (dose required to control p% of irradiated tumors) is a function of radiosensitivity of such cell lines, i.e., m and D0, and of number of cells existing in the tumor (M). The relationship developed from multi-target survival curve is as follows (3):

\[ \text{TCD}_p = D_0 \left( \ln m + \ln M - \ln \ln \frac{100}{p} \right) \]

If 3,000 rads is an enough dose in a single fraction to sterilize a human cancer, the radiosensitivity and number of cells in the tumor could be stated in several figures. For examples, in case of 1) D0=140 rads, m=2, and M=10^8: 2) m=3, D0 =155 and M=10^7 and so on, TCD90 would be around 3,000 rads. We have to remind that animal tumors contain 1-10% hypoxic cells and a tumor of 1 cm³ may contain 5×10^8 tumor cells (providing that cell diameter is 10 μ and half a tumor is composed of tumor cells). If we assume 10% viability in the tumor, 5×10⁷ cells should be killed by radiation. However, TCD90 of 3,000 rads by one exposure corresponds to the sterilization of 10⁸ hypoxic cells with D0=300 rads and m=2 or 3. If 10⁸ hypoxic cells correspond to one percent of total cells in the tumor, only 10⁸ viable cells may exist in the tumor, i.e., very few cells comparing with 5×10⁷ cells as mentioned above. In the other words, killing of 10⁸ cells might be considered as sterilization of the tumor while it contains 5×10⁷ viable cells. There is another question what happened in remaining cells. One should realize that, if we assume more resistant hypoxic cell lines, say D0=400 rads, TCD90 of 2,500 rads by one dose can sterilize a tumor containing 27 such cells. (Several examples of dose-fractionation schema based on multi-target response law are presented in Fig. 1. Also see Appendix where formulae for calculation of these data are presented).

TCD90 of 10⁸ well-oxygenated cells by 30 fractions would be 7,800 rads as calculated by Suit et al. If we assume a less extrapolation number for such cells,
TCD_{90} by 30 fractions would be 6,000 rads. If we further assume that 1% of cells are oxygen-deficient cells (10^6 cells) with radiosensitivity of D_0=300 rads and m=2, 5,000 rads would be required to control 90% of tumors in a single exposure. This is much higher than the clinical dose of 3,000 rads (single exposure). Cell lines of our assumption (D_0 is 300 rads and m is 2), are relatively sensitive compared to the hypoxic cells reported. One can realize an inconsistency between these calculations and clinical data, if 10^8 or 5\times10^7 total (viable) cells were estimated in a tumor. If we assume the presence of this cell number in a tumor, the tumor should contain less hypoxic cells, say 0.001%, i.e., unbelievably small population.

Rather than accepting this situation, we should remind the previous case of

**Fig. 1a-d.** TCD_{90} or 90% tumor control dose, as a function of fractionation number for multi-target theory.
10^5 total cells in which one per cent are hypoxic and TCD_{90} by a single exposure is 3,000 rads. If m=2, 90% of tumors would be killed by 6,300 rads in 30 fractions without any reoxygenation throughout the radiotherapy course. If m is three, 10,000 rads are required to obtain 90% probability of tumor control. However, if all the hypoxic cells are reoxygenized during the 30 fractions, TCD_{90} should be the same as that of 10^5 well-oxygenated cells (e.g., D_0=160 rads) and it might be 6,100 rads (see Fig. 2 for detail. These data are calculated graphically according to the dose-response curve of multi-component cell populations such as reported by Powers and Tolmach).

Summarizing these rethinking of hypoxic cells in the tumor, following two provisional considerations might be implied.

(1) Three thousand rads by a single dose are able to control a tumor containing no more than 10^3 hypoxic cells. If 10^8 viable cells exist in a tumor and all the cells should be killed by ionizing radiation to sterilize the tumor, unbelievable less percentage, i.e., 0.001% of cells are originally hypoxic.

(2) If we assume more practical percentage of hypoxic cells in a tumor, i.e., 1-10%, radiotherapists cannot kill any more than 10^5 cells out of 10^8 viable cells in the tumor. Therefore, there is another question what happened in remaining cells. Are they sterilized by an indirect effect of radiation, e.g., tumor bed effect? Recent data indicate TD_{90} (number of cells expected to induce a tumor in half of the recipients) is the same in both normal and previously irradiated tumor beds. Are they killed by some other factors, e.g., immunologic reaction? Do they have a division probability of less than one? (In this case one can more easily solve this
inconsistency than the other situations assumed.) Or are they just sleeping in?

Lethally irradiated cells which have a stimulating effect to viable tumor cells when they were transplanted in the admixture with viable cells\(^6\), make this inconsistency more complexed. Our recent study\(^7\) indicates that nearly one cell or very few cells might be enough to induce recurrence after the radiotherapy. Therefore, the effect of lethally irradiated cells cannot explain this inconsistency.

Reoxygenation of oxygen-deficient cell population is now well established in several studies of animal tumors.\(^8,9,10\) In human cancer, hypoxic cells might be reoxygenized throughout the radiotherapy. One could realize that almost all the considerations in this paper are based on several assumptions. That means, before we give a conclusion, more experimental and clinical data should be required as pointed out by Alper.\(^11\) It should contain detailed data for division probability of sublethally irradiated cells and of not-irradiated tumor cells. Repair capability of sublethally irradiated cells which were hypoxic at the time irradiated and were in "the tumor" would be another one.

**CONCLUSION**

Radiation dose-fractionation schema was analysed in terms of TCD\(_{90}\) or 90% tumor control dose which is based on multi-target response law. TCD\(_{90}\)'s given in single dose and given in fractionated exposures were calculated on the assumptions that each tumor cell responds to the ionizing radiation independently and it has the similar cellular radiosensitivity as that of cells obtained by many experiments. An inconsistency between these calculated data and clinically obtained data was pointed out and the importance of hypoxic cell population was discussed. Hypoxic cells may not be negligible especially if the single dose exposure is concerned, while clinical data used for, say, 30 fractions would partially be understandable if reoxygenation phenomenon was introduced, as mentioned by Hall.\(^2\) There would be some other factor(s), e.g., small (?) division probability of tumor cells or indirect effect(s) which might play an important role in dose-fractionation schema. This consideration requires more experimental and clinical data.

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**APPENDIX**

Survival fraction \((S)\) after a radiation dose \((D)\) is given in following equation for multi-target theory;

\[
S = 1 - (1 - e^{-D/D_0})^m
\]

where \(D_0\) is a dose required to reduce survival fraction of \(1/e\) in the strait portion of multi-target survival curve and \(m\) is a target number or an extrapolation
If radiation is given in $v$ fractions and each fractionated dose comprises of an equal dose of $D/v$ which is given after the complete cellular recovery from the sublethal damage, the surviving fraction of the cell will be:

$$S_v = \left( 1 - (1-e^{-D/v/D_0})^v \right)$$

Probabilty of the cell death after the fractionated doses:

$$P_v = 1 - S_v$$

If $M$ cells are contained in a tumor and each cell responds to radiation independently, probability of killing $M$ cells ($P$) is given in an equation:

$$P = (1 - S_v)^M$$

If it is assumed that one surviving cell or one cell with the reproductive integrity can produce tumor regrowth, or that all tumor cells are required to receive the lethal radiation damage for the tumor cure, $TCD_p$ will be solved in following formula:

$$P = (1 - (1-e^{-TCD_p/D_0/D_0})^v)^M$$

REFERENCES