Model Tumor Systems

Chapter 21

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Radiobiology for the Radiologist

Introduction

- Model tumor systems are needed to assess effectiveness of various treatments, fractionations, etc.
- A wide range of tumors of different histologic types can be grown in laboratory animals and propagated by transplantation
- Transplanted tumor systems can be highly quantitative
- In general, the more quantitative the system, the more artificial it is, because the tumors are highly undifferentiated and encapsulated

Transplantable tumor systems

- A wide range of tumors of different histologic types can be grown in laboratory animals
- Propagation by transplantation is used in order to produce a large number of virtually *identical* tumors
- The variability among experimental animals has to be minimal (pure inbred strains are maintained)

Transplantable tumor systems

- The tumor from a donor animal is removed and, if possible, prepared into a single-cell suspension by separating the cells with an enzyme such as trypsin and then forcing them through a fine wire mesh
- 10^4 to 10^6 cells are inoculated subcutaneously into each recipient animal
- Some tumors must be propagated by transplanting a small piece of tumor; this is less quantitative
- Within days or weeks, palpable tumors appear in the recipient animals that are uniform in size, type, etc.

Commonly used techniques

There are five commonly used techniques to assay the response of solid tumors to a treatment:

1. Tumor growth measurements
2. Tumor cure (TCD_{50}) assay
3. Tumor cell survival determined *in vivo* by the dilution assay technique
4. Tumor cell survival assayed by the lung colony assays
5. Tumor cell survival using *in vivo* treatment followed by *in vitro* assay

Apoptosis in tumors

- Apoptosis occurs in both normal tissues and tumors, spontaneously and as a result of irradiation
- If a tumor responds rapidly to a relatively low dose of radiation, it generally means that apoptosis is involved, since the process peaks at 3 to 5 hours after irradiation
- Susceptibility to the induction of apoptosis also may be an important factor determining radiosensitivity
- Apoptosis was found to be most important in lymphomas, essentially absent in sarcomas, and intermediate and variable in carsinomas

Sarcoma (from greek "flesh") – cancer originating in connective tissues, e.g., muscles, bones, fat
Carcinoma – originates from epithelial cells
Lymphoma – cancer of lymphatic system cells, lymphocytes (either B or T type)

Tumor growth measurements

- The simplest technique to use
- Involves the daily measurement of each tumor to arrive at a mean diameter
- A large number of transplanted tumors are prepared
- When tumors have grown to a specified size (e.g., a diameter of 8 to l0 mm in rats or 2 to 4 mm in mice), they are treated according to the plan of the particular experiment

Tumor growth measurements

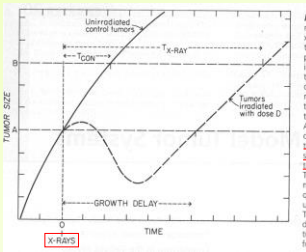


FIGURE 20.1 ● The pattern of response of a tumor to a dose of x-rays. The size of the tumor, either the mean diameter or the volume, is plotted as a function of time after irradiation. Two different indices of tumor responses have been used by different investigators. Growth delay represents the time after irradiation that it takes for the tumor to regrow to the size at the time of irradiation. Alternatively, the index of radiation damage may be the time taken for the tumor to grow from a specified size A at the time of irradiation to some specified larger size B. Typically, this may be from 9 to 25 mm in diameter for rat tumors. This quantity is shown as T_{CON} for unirradiated control animals and $T_{X\text{-}RAY}$ for tumors irradiated with a dose (D) of x-rays. Either index of tumor response may be plotted as a function of radiation dose.

- The untreated tumors grow rapidly at a relatively uniform rate
- The radiation treatment causes a temporary shrinkage of the tumor, followed by regrowth

Tumor growth measurements

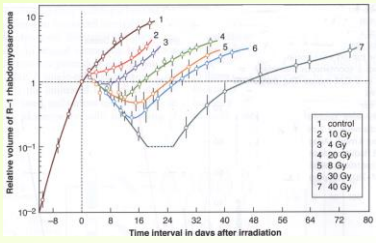


FIGURE 21.3 A: Volume changes of rhabdomyosarcomas (cancer of skeletal muscles) in rats after irradiation. Curve 1 represents the growth of the unirradiated control tumors. Curves 2,4,6, and 7 refer to tumors irradiated with 10 to 40 Gy of 300-kV x-rays. Curves *3* and 5 refer to tumors irradiated with 4 and 8 Gy of 15-MeV d^+ —> T fast neutrons

Tumor growth measurements

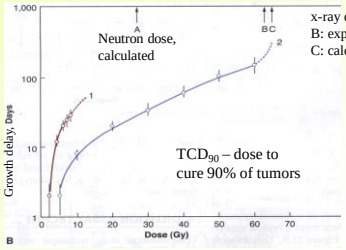


Figure 21.3 **B:** Growth delay of rhabdomyosarcomas in rats as a function of dose of x-rays (curve 2) or fast neutrons (curve 1). A and C indicate the doses of neutrons and x-rays, respectively, required to "cure" 90% of the tumors, calculated on the basis of cell survival curves. B indicates the observed TCD_{90} for x-rays. Note the good agreement between calculated and observed values of the TCD_{90} for x-rays. (From Barendsen GW, Broerse JJ: Experimental radiotherapy of a ra

Tumor cure (TCD_{50}) assay

- Data most relevant to radiation therapy
- A large number of animals with tumors of uniform size are divided into separate groups, and the tumors are irradiated locally with graded doses
- The proportion of tumors that are locally controlled can be plotted as a function of dose
- TCD_{50} – the dose at which 50% of the tumors are locally controlled; this quantity is highly repeatable from one experiment to another in an inbred strain of animals

Tumor cure (TCD_{50}) assay

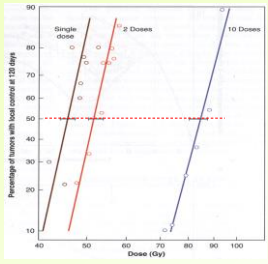


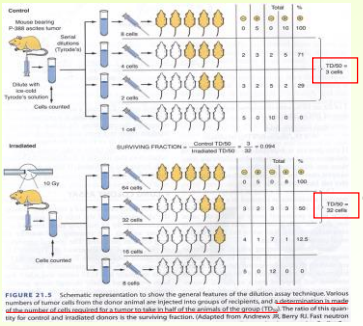
FIGURE 21.4 Percentage of mouse mammary tumors locally controlled as a function of x-ray dose for single exposures and for two different fractionation patterns. The tumors were isotransplants derived fnom a spontaneous mammary carcinoma in a C_3H mouse. The trans-plantation was made into the outer portion of the ear with $4X10^4$ viable cells. The tumors were treated when they reached a diameter of 2 mm (i.e., a volume of about 4 mm^3)

- A brass circular clamp was fitted across the base of the ear and maintained for at least a minute before the initiation of the irradiation, so that the tumors were uniformly hypoxic
- Single-dose, 2-dose, and 10-dose experiments were performed, with a 24-hour interval between dose fractions
- An extensive repair of sublethal damage has taken place during a multifraction regimen

Dilution assay technique

- Devised by Hewitt and Wilson, who used it to produce the first *in vivo* cell survival curve in 1959
- A single-cell suspension can be prepared from the infiltrated liver of an animal with advanced lymphocytic leukemia disease
- The tumor transplanted by injecting known numbers of cells into the peritoneal cavities of recipient mice, which subsequently develop leukemia (it can be transmitted, on average, by the injection of only two cells)
- The number of cells required to transmit the tumor to 50% of the animals - is known as TD_{50}

Dilution assay technique

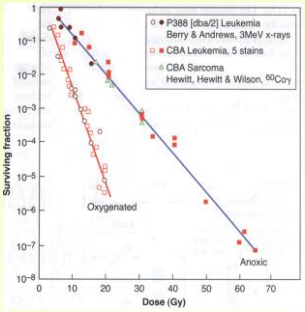


TD_{50} controls

TD_{50} irradiated

Surviving fraction = TD_{50} controls/ TD_{50} irradiated

Dilution assay technique



The technique is applicable to solid tumors as well

FIGURE 21.6 Dose-response curves *in vivo*, using the dilution assay technique, for various murine tumors under oxygenated and hypoxic conditions. (Adapted from Berry RJ. On the shape of x-ray dose-response curves for the reproductive survival of mammalian cells. *Br J Radiol.* 1964;37:948-951, with permission.)

Lung colony assay

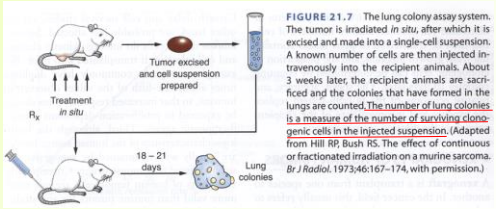


FIGURE 21.7 The lung colony assay system. The tumor is irradiated *in situ*, after which it is excised and made into a single-cell suspension. A known number of cells are then injected intravenously into the recipient animals. About 3 weeks later, the recipient animals are sacrificed and the colonies that have formed in the lungs are counted. The number of lung colonies is a measure of the number of surviving clonogenic cells in the injected suspension. (Adapted from Hill RP, Bush RS. The effect of continuous or fractionated irradiation on a murine sarcoma. *Br J Radiol.* 1973;46:167–174, with permission.)

- Technique to assay the clonogenicity of the cells of a solid tumor irradiated *in situ* (*in vivo*) by injecting them into recipient animals and counting the number of lung colonies produced
- Can be used with different tumor cells

In vivo/in vitro assay

- A limited number of cell lines have been adapted so that they grow *either* as a transplantable tumor in an animal *or* as clones in a petri dish
- These cells can be readily transferred from *in vivo* to *in vitro* and back. In one generation they may grow as a solid tumor in an animal, and in the next as a monolayer in a petri dish
- The three most commonly used systems are a rhabdomyosarcoma in the rat (Hermens and Barendsen), a fibrosarcoma in the mouse (Mc-Nally), and the EMT6 mammary tumor, also in the mouse (Rockwell and Kallman)

In vivo/in vitro assay

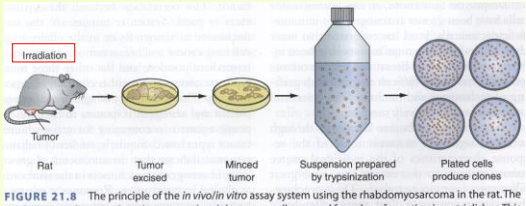


FIGURE 21.8 The principle of the *in vivo/in vitro* assay system using the rhabdomyosarcoma in the rat. The solid tumor in the animal can be removed and the tumor cells assayed for colony formation in petri dishes. This cell line can be transferred back and forth between the animal and the petri dish. (Adapted from a drawing courtesy of Drs. G.W. Barendsen and J.J. Broerse.)

The speed, accuracy, and relative economy of the *in vitro* system replaces the expense and inconvenience of the recipient animals in the dilution assay technique

Xenografts of human tumors

- A **xenograft** is a transplant from one species to another; usually refers to a human tumor growth transplanted in a laboratory animal
- If the recipient animal has a normal immune system, a xenograft should not grow, but there are two main ways in which growth has been achieved:
  - Animal strains have been developed that are congenitally immune deficient (nude mice, nude rats, and SCID mice)
  - It is possible to severely immune-suppress mice by the use of radiation or drugs or a combination of both
- Neither type of host completely fails to reject the human tumor cells, complicating the interpretation

Xenografts of human tumors

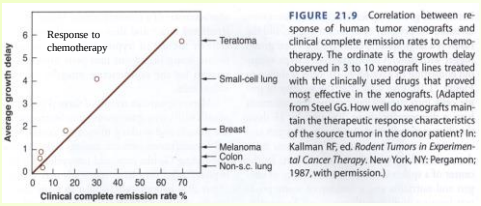


FIGURE 21.9 Correlation between response of human tumor xenografts and clinical complete remission rates to chemotherapy. The ordinate is the growth delay observed in 3 to 10 xenograft lines treated with the clinically used drugs that proved most effective in the xenografts. (Adapted from Steel GG. How well do xenografts maintain the therapeutic response characteristics of the source tumor in the donor patient? In: Kallman RF, ed. *Rodent Tumors in Experimental Cancer Therapy*. New York, NY: Pergamon; 1987, with permission.)

- More than 300 tumors were investigated by this technique
- Xenografts retain human genomes and maintain some of the response characteristics of the individual source human tumors

Xenografts of human tumors

- There are certain drawbacks:
  1. There is a tendency for the tumor to be rejected, so that observing tumor control can be misleading. Growth delay and cell survival studies are probably less affected
  2. Human tumor cells do undergo kinetic changes and cell selection if transplanted into mice. For example, xenografts commonly have doubling times about 1/5th of the values observed in humans, so that increased responsiveness is expected to proliferation-dependent chemotherapeutic agents
  3. They are not valid for any studies in which the vascular supply plays an important role
- Generally, there is an agreement between patient (clinical responsiveness) and xenograft responses to chemotherapeutic agents and radiation

Spheroids: *in vitro* model tumor systems

- Mammalian cells in culture may be grown either as a monolayer attached to a glass or plastic surface or in suspension with continual gentle stirring. Some established rodent cells instead grow as spheroids
- Oxygen and nutrients must diffuse into the spheroids from the surrounding tissue culture medium
- In the center of a spheroid, there is a deficiency of oxygen and nutrients and a buildup of waste products because of diffusion limitations
- Eventually, central necrosis appears and the mean cell cycle lengthens

Spheroids: *in vitro* model tumor systems

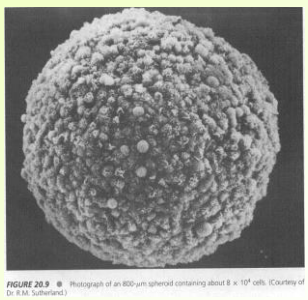
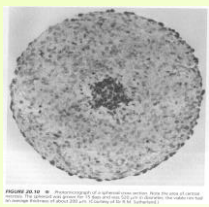
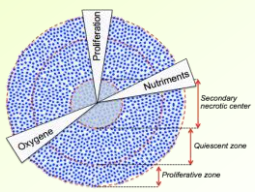


FIGURE 20.9 Photograph of an 800-μm spheroid containing about 8×10^6 cells. (Courtesy of Dr. R.M. Sutherland.)

- Five days after the seeding of single cells into suspension culture, the spheroids have a diameter of about 200 μm; by 15 days, the diameter may exceed 800 μm
- Mature spheroids contain a heterogeneous population of cells resulting from many of the same factors, as in a tumor *in viro*
- More quantitative and more economical to work with than tumors

Spheroids of human tumor cells





http://ip3d.itav-recherche.fr/IP3D/Spheroids_imaging.html

- Mature spheroids consist of three populations of cells with varying radiosensitivity. From the outside and toward the center they are:
  - asynchronous, aerobic cycling cells,
  - aerated noncycling G_1-like cells, and
  - noncycling G_1-like hypoxic cells

Use of tumor models

- Transplantable tumors in laboratory animals are model systems
- Transplantable tumors tend to be fast growing, undifferentiated, and highly antigenic and are grown as encapsulated tumors in muscle or beneath the skin, not in their sites of origin
- For all of these reasons, they are highly artificial, and care must be used in interpreting results

Cell, Tissue, and Tumor Kinetics

Chapter 22

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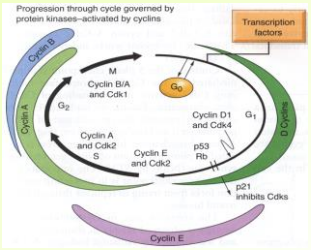
The cell cycle

- Identification of phases of a cell cycle, M, G1, S, and G2, was accomplished in the 1950s
  - M phase can be identified with light microscope, S with autoradiography
  - Between M and S phases was the "first gap in activity" (G_1), and between S phase and the next M was the "second gap in activity" (G_2)
- If the cells stop progressing through the cycle (if they are arrested) they are said to be in G_0
- Cell may arrest its cycle in G phases after low dose of radiation
- Tumor cells often show only G_2 arrest point

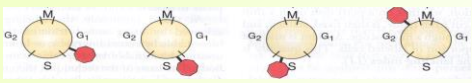
Cyclins and kinases in cell cycle



Transitions in the cycle occur only if a given kinase activates the proteins required for progression

- Progression through the cell cycle is governed by protein kinases, activated by cyclins
- In mammals, cyclins A through H have been described. Each cyclin protein is synthesized at a discrete phase of the cycle: cyclin D and E in G_1, cyclin A in S and G_2, and cyclin B in G_2 and M

Checkpoint pathways



- Events in the cell cycle must take place in a specific order, and it is the function of a number of checkpoint genes to ensure that the initiation of late events is delayed until earlier events are complete
- Normal cells with damaged DNA stop progressing through the cycle; there are 3 principal places at which checkpoints function:
  1. G_1/S checkpoint
  2. S phase checkpoint
  3. G_2/M checkpoint

Quantitative assessment of cell cycle

- The proportion of cells that are seen to be in mitosis is counted; this quantity is called the **mitotic index** (MI). Assuming that all of the cells in the population are dividing and that all of the cells have the same mitotic cycle length T_C,

$$MI = \lambda T_M / T_C$$

- If the cell population is fed for a brief time with tritiated thymidine or 5-bromodeoxyuridine (**flash-labeled)**, cells get labeled during the S phase. A count is made of the proportion of labeled cells. The **labeling index** (LI) is defined as:

$$LI = \lambda T_S / T_C$$

- Both quantities can be determined from a single specimen

Percent labeled mitoses technique

- The technique is laborious and time-consuming and requires a large number of serial samples
- It is readily applicable *in vitro*, for which it is not difficult to obtain a large number of replicate samples
- It also may be applied *in vivo* for determining the cell-cycle parameters of normal tissue or tumors, provided a large number of sections from matched animals or tumors can be obtained at accurately timed intervals

Percent labeled mitoses technique

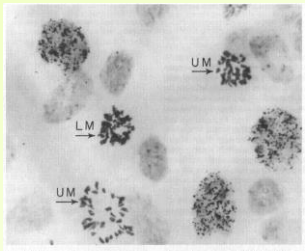


FIGURE 21.3 ● Photomicrograph of a preparation of mouse corneal cells. The cell preparation was flash-labeled some hours before with tritiated thymidine, which was taken up by cells in S phase. By the time the autoradiograph was made, the cell marked *LM* had moved around the cycle into mitosis; this is an example of a labeled mitotic figure. Other cells in mitosis are unlabeled (*UM*). (Courtesy of Dr. M. Fry.)

- Only 1 to 2 % of the cells are in mitosis, and only a fraction of these will be labeled
- The assumption is made that all cells have identical cycles

Percent labeled mitoses technique

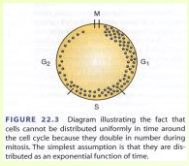


FIGURE 22.3 Diagram illustrating the fact that cells cannot be distributed uniformly in time around the cell cycle because they double in number during mitosis. The simplest assumption is that they are distributed as an exponential function of time.

- Cells in S phase take up the radioactive label
- After the label is removed, cells progress through their cell cycles
- Every hour a sample is removed, and a radioactive label must then be counted; this is the **percentage of labeled mitoses**

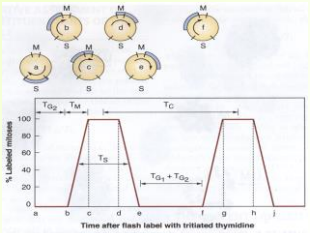


FIGURE 22.5 Percent-labeled mitoses curve for an idealized cell population in which all of the cells have identical mitotic cycle times. The cell population is flash-labeled with tritiated thymidine, which labels all cells in S. The proportion of labeled mitotic cells is counted as a function of time after labeling. The circles at the top of the figure indicate the position of the labeled cohort of cells as it progresses through the cycle. The length of the various phases (e.g., T_G, T_M) of the cycle (T_C) may be determined as indicated.

Percent labeled mitoses technique

- The total cycle (Tc) is the distance between corresponding points on the first and second wave or the distance between the centers of the two peaks. The remaining quantity, T_{G1} , usually is calculated by subtracting the sum of all the other phases of the cycle from the total cell cycle, or

$$T_{G1} = T_C - (T_S + T_{G2} + T_M)$$

- Experimental data are never as clear-cut as the idealized picture, especially if the population of cells involved is an *in vivo* specimen of a tumor or normal tissue

Percent labeled mitoses technique

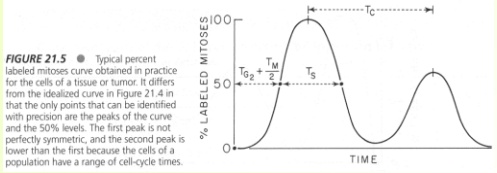


FIGURE 21.5 ● Typical percent labeled mitoses curve obtained in practice for the cells of a tissue or tumor. It differs from the idealized curve in Figure 21.4 in that the only points that can be identified with precision are the peaks of the curve and the 50% levels. The first peak is not perfectly symmetric, and the second peak is lower than the first because the cells of a population have a range of cell-cycle times.

- The T_S is given approximately by the width of the first peak, at the 50% level
- The total cell cycle T_C is the time between successive peaks
- In a separate experiment can find MI= $\lambda T_M/T_C$
- The time from flash labeling to the 50% level is T_{G2} + 0.5 T_M
- The remaining T_{G1} is found from the total cycle time

Measurements of cell cycle times

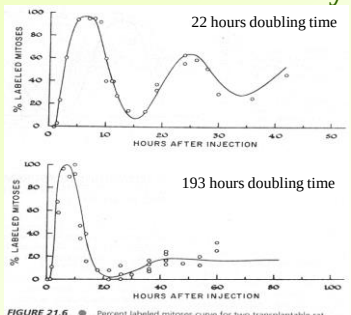


FIGURE 21.6 ● Percent labeled mitoses curve for two transplantable rat sarcomas with widely different growth rates. The tumor in the **upper panel** has a gross doubling time of 22 hours, compared with 192.5 hours for the tumor in the **lower panel**. (From Steel GG, Adams K, Barratt JC: Analysis of the cell population kinetics of transplanted tumours of widely differing growth rate. *Br J Cancer* 20:784–800, 1966, with permission.)

Need additional information, labeling index LI, to determine T_C

Measurements of cell cycle times

TABLE 21.1

The Constituent Parts of the Cell Cycle (in hours) for Some Cells in Culture and Tumors in Experimental Animals

Authors	Cell or Tissue	T_C	T_S	T_M	T_{G_2}	T_{G_1}
Bedford	Hamster cells *in vitro*	10	6	1	1	2
	HeLa cells *in vitro*	23	8	1	3	11
Steel	Mammary tumors in the rat					
	BICR/M1	19	8	~1	2	8
	BICR/A2	63	10	~1	2	50
Quastler and Sherman	Mouse intestinal crypt	18.75	7.5	0.5	0.5–1.0	9.5
Brown and Berry	Hamster cheek pouch epithelium	120–152	8.6	1.0	1.9	108–140
	Chemically induced carcinoma in pouch	10.7	5.9	0.4	1.6	2.8

• Although the length of the cell cycle varies enormously between populations, the lengths of G2, mitosis, and S are remarkably constant
• It usually is found that the malignant cells have the shorter cycle time than the normal-tissue cells

Pulsed flow cytometry

- Provides data within a few days
- Cells are stained with a DNA-specific dye and sorted on the basis of DNA content
- Detailed cell kinetic data can be obtained by such techniques, including an analysis of the distribution of cells in the various phases of the cycle
- In practice, the measurement of most immediate relevance to clinical radiotherapy is the estimate of T_{pot}, the **potential tumor doubling time**

Pulsed flow cytometry

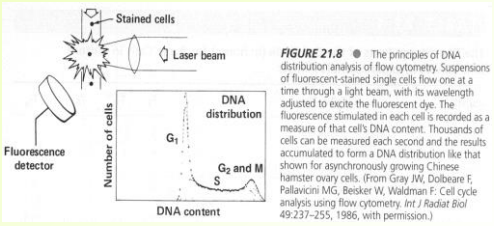


FIGURE 21.8 ● The principles of DNA distribution analysis of flow cytometry. Suspensions of fluorescent-stained single cells flow one at a time through a light beam, with its wavelength adjusted to excite the fluorescent dye. The fluorescence stimulated in each cell is recorded as a measure of that cell's DNA content. Thousands of cells can be measured each second and the results accumulated to form a DNA distribution like that shown for asynchronously growing Chinese hamster ovary cells. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. *Int J Radiat Biol* 49:237–255, 1986, with permission.)

The cells are stained simultaneously with two dyes that fluoresce at different wavelengths: One binds in proportion to DNA content to indicate the phase of the cell cycle, and the other binds in proportion to the amount of dye incorporation to show if cells are synthesizing DNA

Measurement of Tpot

- T_{pot} is a measure of the rate of increase of cells capable of continued proliferation; it may determine the outcome of a radiotherapy treatment protocol delivered in fractions over an extended period of time
- Precise determination requires measurement of T_S and labeling index LI, Tpot= λT_S/LI
- Estimate of Tpot can be made by the flow cytometry from a single biopsy specimen taken 4 to 8 hours after the injection of a thymidine analogue
- A single-cell suspension of the biopsy specimen is then passed through a flow cytometer, which simultaneously measures DNA content (red) and a thymidine analogue content (green)

Measurement of Tpot

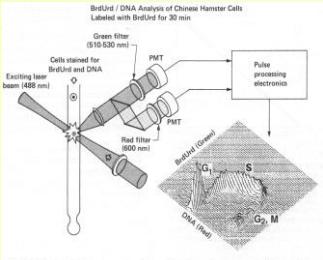


FIGURE 21.9 ● The flow cytometric analysis of cellular bromodeoxyuridine (BrdUrd) and DNA content for cells stained with fluorescein (linked to BrdUrd) and propidium iodide (linked to DNA). The cells are processed one at a time through a blue (488-nm) laser beam that excites cellular BrdUrd content, and red fluorescence is recorded as a measure of cellular DNA content. The BrdUrd (green fluorescence) axis in the biwariate is logarithmic, with every seven channels representing a doubling of fluorescence intensity. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. *Int J Radiat Biol* 49:237–255, 1986, with permission.)

- LI is the proportion of cells that show significant green fluorescence
- Ts can be calculated from the mean red fluorescence of S cells relative to G_1 and G_2 cells. The DNA content of cells in G_2 is double that in G_1.
- The method assumes that the red fluorescence of bromodeoxyuridine-labeled cells (i.e., the DNA content of cells in S phase) increases linearly with time
- This technique gives an average value for T_{pot} of the cells in the biopsy specimen

Growth fraction

- At any given moment, some tumor cells are proliferating (P) and some are quiescent (Q)
- Growth fraction is defined as GF=P/(P+Q)
- Experimentally can be found through cell labeling, with autoradiograph taken several generations after injection:

$$GF = \frac{\text{fraction of cells labeled}}{\text{fraction of mitoses labeled}}$$

Growth fraction

TABLE 21.2

Growth Fraction for Some Tumors in Experimental Animals

Tumor	Author	Growth Fraction, %
Primary mammary carcinoma in the mouse (G_3H)	Mendelsohn	35–77
Transplantable sarcoma in the rat (RIB_5)	Denekamp	55
Transplantable sarcoma in the rat (SSO)	Denekamp	47
Transplantable sarcoma in the rat (SSB_1)	Denekamp	39
Mammary carcinoma in the mouse (C_3H)	Denekamp	30
Chemistry induced carcinoma in the hamster cheek pouch	Brown	29

- GFs measured for a variety of solid tumors in experimental animals frequently fall between 30 and 50%
- As a tumor outgrows its blood supply, it develops areas of necrosis and hypoxic cells, the proportion of which for many solid tumors is about 15%
- This still does not account for all quiescent cell population

Cell loss

- In most cases, tumors grow much more slowly than would be predicted from a knowledge of the cycle lime of the individual cells and the growth fraction; the difference is a result of cell loss
- Cell loss factor Φ=1-Tpot/Td, where Td is the tumor doubling time obtained from direct measurements of the tumor volume
- The cell loss factor represents the *ratio of the rate of cell loss to the rate of new cell production.* A cell loss factor of 100% indicates a steady state of neither growth nor regression

Cell loss

1. Death from inadequate nutrition as the tumor outgrows its vascular system some cells experience an inadequate concentration of oxygen and other nutrients
2. Apoptosis, or programmed cell death: this form of cell death is manifested by the occurrence of isolated degenerate nuclei remote from regions of overt necrosis
3. Death from immunologic attack
4. Metastasis, including all processes by which tumor cells are lost to other parts of the body, such as spread through the bloodstream and lymphatic system
5. Exfoliation, for example, in carcinoma of the gastrointestinal tract, in which the epithelium is renewed at a considerable rate

Cell loss in experimental animal tumors

TABLE 21.3

Cell Loss Factor (Φ) for Some Tumors in Experimental Animals

Tumor	Author	Φ, %
Mouse sarcoma	Frindel	
3-day-old tumor		0
7-day-old tumor		10
20-day-old tumor		55
Rat carcinoma	Steel	9
Rat sarcoma	Steel	0
Mouse carcinoma	Mendelsohn	69
Hamster carcinoma	Brown	75
Rat sarcoma	Hermens	26
Hamster carcinoma	Reiskin	81–93
Mouse carcinoma	Tannock	70–92

- Values for the cell loss factor vary from 0 to more than 90%
- All the sarcomas investigated had cell loss factors less than 55%; most carcinomas - in excess of 70%
- In the long term, the "cure" rates of both tumors may well be identical, but in the short term, the carcinoma would be said to have "responded", whereas the sarcoma might be said to be resistant to radiation

Growth kinetics in human tumors

- Tumors of the same histologic type arising in different patients differ widely in growth rate
- Metastases arising in the same patient tend to have similar rates of growth
- There is a correlation between histologic type and growth rate
- The degree of differentiation seems to be related to the doubling time, with poorly differentiated cancers generally progressing more rapidly

Growth kinetics in human tumors

TABLE 21.4

Individual Values for the Duration of the Cell Cycle (T_C) in Human Solid Tumors of Various Histologic Types

Authors	T_C, h
Frindel et al. (1968)	97, 51.5, 27.5, 48, 49.8
Bennington (1969)	15.5, 14.9
Young and de Vita (1970)	42, 82, 74
Shirakawa et al. (1970)	120, 144
Weinstein and Frost (1970)	217
Terz et al. (1971)	44.5, 31, 14, 25.5, 26
Peckham and Steel (1973)	59
Estevez et al. (1972)	37, 30, 48, 30, 38, 96, 48
Terz and Curutchet (1974)[a]	18, 19, 19.2, 120
Malaise et al. (unpublished data)[a]	24, 33, 48, 42
Muggia et al. (1972)	64
Bresciani et al. (1974)	82, 50, 67, 53, 58

[a] Measured by the mean grain count halving time.
From Tubiana M, Malaise E: Growth rate and cell kinetics in human tumors: Some prognostic and therapeutic implications. In Symington T, Carter RL (eds): *Scientific Foundations of Oncology*, pp 126–136. Chicago, Year Book Medical Publishers, 1976, with permission.

- Studies of cell population kinetics also have been performed on a limited number of human tumors
- Studies of this kind raise practical and ethical problems
- In humans, each spontaneous tumor is unique, so the multiple samples must be obtained by repeated biopsies at frequent intervals from the same tumor

Growth kinetics in human tumors

TABLE 21.5

Volume-Doubling Times of Human Tumors

Authors	Site	Volume-Doubling Time, d	Range, d
Breuer	Lung metastases	40	4–745
Collins et al.	Lung metastases	40	11–164
Collins	Lung metastases from colon or rectum	96	34–210
Garland	Primary bronchial carcinoma	105	27–480
Schwartz	Primary bronchial carcinomas	62	17–200
Spratt	Primary skeletal sarcomas	75	21–366

Based on data from Steel GG: Cell loss from experimental tumors. *Cell Tissue Kinet* 1:193–207, 1968.

- Although the tumor doubling time is characteristically 40 to 100 days, the cell-cycle time is relatively short, 1 to 5 days
- As a general rule, the histologic groups of human tumors that have the most rapid mean growth rates and the highest growth fractions and cell turnover rates are those that are the most sensitive to radiation and chemotherapy

Growth kinetics in human tumors

- In 90% of human tumors, the cell-cycle time has a modal value of 48 hours (a range of 15 to 125 hours)
- In human tumors, Ts has a modal value of about 16 hours (a range of 9.5 to 24 hours)
- As a first approximation, the mean duration of the cell cycle in human tumors is about three times the duration of the S phase
- Growth fraction is more variable in human tumors than in rodent tumors and correlates better with gross volume-doubling time
- Cell loss factor for human tumors has been estimated by Tubiana and Malaise to have an average value for a range of tumors in excess of 50%. Steel's estimate for a median value for all human tumors studied is 77%

Summary

- Model tumor systems are used for evaluation of tumor response to different treatments. A wide range of different hystologic types can be grown and propagated by transplantation
- The five assays in common use are tumor growth delay measurements, tumor cure (TCD_{50}) assay, tumor cell survival determined by the dilution assay technique, the production of lung colonies, and *in vivo* treatment followed by *in vitro* assay
- Growth kinetics of tumors can be assessed quantitatively through studies of cell cycles through labeling techniques
- Different aspects can be taken into account in designing the most effective treatment regimen