Cell, Tissue and Tumor Kinetics

Proliferation Kinetics: rate of growth of a population, change in total cell number. Adult tissues are in homeostasis. Children (and tumors) grow.

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I. Quantitative Assessment of Parts of the Cell Cycle

Mitotic Index (MI): proportion of cells in mitosis (count directly)

$$\mathbf{MI = T_M/T_C}$$

where:
T_M = time for mitosis
T_C = total cell cycle time

Assumes:

- All cells in the population are dividing
- All cells have the same cell cycle times
- Cells are uniformly distributed around the cell cycle (probably not)

[Image removed due to copyright considerations]

Labeling Index (LI): proportion of cells in S phase

$$\mathbf{LI = T_S/T_C}$$

- Same assumptions as for MI.
- Can be determined by **pulse-labeling** (10-30 min) cells with DNA precursors (^{3}H-thymidine or with BrdUrd), fixing, staining as appropriate and counting labeled cells.
- Note that both MI and LI are ratios, not actual duration of phases of the cell cycle.

Percent-labeled-mitoses.

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Label cells in one part of the cell cycle.

Observe passage of these cells through another part of the cell cycle.

Requires staining and scoring multiple samples from the original population.

b first labeled mitotic figures appear
ab = T_{G2}
bc = T_M
bd = T_S = ce
T_C = peak 1 - peak 2
T_{G1} = $T_C – (T_S – T_{G2} – T_M)$

[Image removed due to copyright considerations]

Real data shows asymmetric peaks.

Typical values for duration of parts of cell cycle:

T_C 10 h–10 days
T_{G1} 1–150 h
T_S 6-10 h
T_{G2} 1-2 h
T_M 1 h

Cell population has a variety of cell cycle times.

If T_C is long, variability is greater; second peak may not be distinct.

TABLE 21.1. *The Constituent Parts of the Cell Cycle for Some Cells in Culture and Tumors in Experimental Animals*

Authors	Cell or Tissue	T_C, h	T_S, h	T_M, h	T_{G2}	T_{G1}
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

- T_S is similar in all cell lines
- T_{G1} varies widely and is the major contributor to the widely different T_C values observed

Growth Fraction (GF)

Not all cells in a tumor are actively growing.

- Proliferating cells (P)
- Quiescent cells (Q)

Growth fraction is ratio of the number of proliferating cells (P) to the total number of cells.

GF = P/(Q+P)

- Determined by continuous labeling of cells for ~one cell cycle time (T_C).
- GF = fraction of cells labeled.
- Growth fraction for experimental animal tumors is usually 30-50%.

Or use of antibody (Ki-67) that binds to a nuclear antigen in cycling cells.

Because not all cells in a tumor are in the growth fraction, the actual (measured) **tumor volume doubling time (T_D)** is usually longer than the cell cycle time.

Normal adult tissues show **no net growth**: growth fraction balanced by **cell loss**.

Flow cytometry

Provides a direct measure of all phases of the cell cycle.

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Potential Doubling Time (T_{pot})

The parameter of most immediate relevance to clinical radiotherapy.

- T_{pot} is the cell cycle time/fraction of cycling cells:

$$\mathbf{T_{pot} = T_C/GF}$$

- T_{pot} does not take cell loss into account

T_S and LI can be determined by the % labeled mitoses approach, but this is not practical in humans.

An average T_{pot} can be estimated for tumors using flow cytometry with the dual fluorescence method:

$$\mathbf{T_{pot} = (T_S/LI)}$$

- Inject a tracer amount of BrdUrd
- 4-8 hours later obtain tumor biopsy
- Stain with antibody to BrdUrd (green)
- Stain DNA with propidium iodide (red)
- Disaggregate for flow cytometry analysis.

[Image removed due to copyright considerations]

T_{pot} is being studied as a potential predictive assay.

Objective is to identify patients whose tumors are growing rapidly.

These tumors might benefit from an accelerated fractionation schedule.

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LI is the proportion of cells that show green fluorescence (BrdUrd labeled).

T_S is calculated from mean red fluorescence of S cells relative to G_1 and G_2 cells (assumes DNA content in S phase cells increases linearly through S phase).

Relative Movement (RM)

RM = (FL-FG1)/(FG2-FG1)

FL = the mean red fluorescence of the BrdUrd labeled cells
FG1 and FG2 are the mean red fluorescence of the G1 and G2 populations

$$T_S = \frac{0.5t}{RM - 0.5}$$

Cell Loss

- Tumors usually grow much more slowly than predicted by knowledge of T_C and GF because of cell loss.
- Tumor growth is the net result of cell growth and cell loss.
- Cells can be lost due to death from:
  - **inadequate nutrition** (necrosis, hypoxia, reflects inability of the vascular supply to keep up with tumor growth)
  - apoptosis
  - immunological surveillance
  - metastasis
  - exfoliation.

Cell loss factor, ϕ:

$$\phi = 1 - (T_{pot}/T_D) \text{ or } T_D = T_{pot}/(1 - \phi)$$

Example:
If T_C = 22h, GF = 0.6, and ϕ = 0.9, the T_D = 366 hrs

- In experimental animal tumors, cell loss factors range from 0 to 90%.
- Carcinomas tending to have high cell loss factors (>70%) and sarcomas tending to have low cell loss factors (<30%).
- This might be attributable to the origin of carcinomas from continuously renewing epithelial tissues, where the cell loss factor is 100%.
- This might also account for the differing responses of sarcomas and carcinomas to radiation.

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Summary:

Potential doubling time $T_{pot} = \frac{T_C}{GF}$ $T_{pot} = \frac{T_S}{LI}$ **(**flow cytometry)

Cell loss $\phi = 1 - \frac{T_{pot}}{T_D}$

Tumor doubling time $T_D = \frac{T_{pot}}{(1-\phi)}$

V. Volume Doubling Time, T_D

Three factors determine tumor growth

- Cell cycle time of proliferating cells
- Growth fraction
- Cell loss fraction

Cell cycle times for tumor cells ($\mathbf{T_C}$) ~ 1-5 days
Tumor volume doubling times ($\mathbf{T_D}$) ~40-100 days

Example:
Initial tumor diameter = 0.5 cm. $V = (4/3)\pi r^3$
100 days later the tumor had grown to 4 cm.
What is the tumor doubling time?

Calculation:

- Diameter increased by a factor of 8.
- $8^3 = 512$-fold increase in tumor volume.
- How many doublings occurred during the 100 days?
- $2^n = 512$;
- n = 9 doublings
- Nine doublings in 100 days = 1 doubling every 11 days

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Relationship between tumor weight, number of cells it contains, and the number of doublings per cell. (assumes 10^9 cells per gram tissue)

Tumor growth tends to be Gompertzian

Doubling time of the tumor increases progressively as the tumor gets bigger.

Cell cycle time is probably constant
This implies that GF, cell loss factor, vary as a function of tumor size.

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Kinetics of Human Tumors

Some parameters

- T_C usually between 15 and 125 h, modal value of 48 h
- T_S usually between 10 and 24 h, modal value of 16 h.
- T_C usually 3 x T_S, so LI gives an approximate value of GF.
- LI varies between 0.1% and 40%, so GF varies between 0.3% and 100%.
- Cell loss factors high, usually in excess of 50%. Thus, cell loss may be the most important factor in determining the pattern of growth of human tumors.
- Volume doubling times range from 18 to over 200 days, with an average median doubling time estimated to be 66 days.

Generally, the cell cycle time of malignant cells is shorter than that of their normal tissue counterparts.

Metastases appear to grow more rapidly than the primaries in the same individual.

Characteristics of different types of tumors

Histological Type	Doubling Time (Days)	Labeling Index (%)	Growth Fraction (%)	Cell Loss (%)	Rate of Cell Renewal Per Day (%)	Radiosensitivity (Mean Dose for Tumor Sterilization) (Gy)	Chemo-sensitivity
Embryonal Tumors	27 (22-33)	30 (22-41)	90	94	42	25-30	++
Lymphomas	29 (23-37)	29 (22-38)	90	94	51	35-40	++
Mesenchymal Sarcomas	41 (35-50)	(2.5-5.9) 8.3	11	68	5.5	85	--
Squamous Carcinomas	58 (48-70)	8.3 (6.4-10.9)	25	90	10	60-70	+
Adenocarcinomas	83 (72-96)	2.1 (1.7-2.7)	6	71	1	60-80	±

Numbers in brackets are 95% confidence limits

From Tubiana *et al.*

- Tumors with high GF tend to be more radiosensitive.
- GF, LI, cell loss factor determine response to chemotherapy (acting on S phase cells)
- Chemotherapy cures observed only in tumors with high LI.

Kinetics After Irradiation

If cells are growing normally and have adequate nutrition, etc. (e.g., exponentially growing cells in culture or in a tumor), immediately after irradiation their cell cycle will increase, largely due to division delay.

- Subsequently, the loss of some cells in a tumor or non-growing normal tissue may cause the remaining cells to show accelerated growth or **repopulation**.

- This may result from shortening of the T_C, increased growth fraction, and/or decreased cell loss.

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Tumor behavior after irradiation will depend, at least in part, on the cell loss factor, GF and T_C of the tumor.

Influence of Kinetic Parameters on Tumor Growth and on Regression After Irradiation (Representative Combinations Only)

ϕ	GF	T_C	Tumor Behavior
Low	Low	Long	Slow growth; slow regression
High	Low	Short	Slow growth; rapid regression
High	High	Short	Rapid growth; rapid regression
Low	High	Long	Rapid growth; slow regression

from Withers and Fletcher in Fletcher, 1980