

Tumour Stem Cells—The Evidence and the Ambiguity

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Progressive growth of malignant tumours, metastatic spread and local recurrence after treatment can only be explained by the presence of cells with unlimited proliferative ability. While this is generally accepted, the proportion of such cells and their organization in a hierarchical system of stem cells and non-stem cell progeny is still a matter of controversy. Results of quantitative transplantation and dose requirement of curative radiotherapy have indicated low stem cell fractions in human and early passage rodent tumours, but uncertainty is introduced by uncontrollable experimental or biological factors and the probabilistic nature of stem cell performance itself. Studies using a particular mouse carcinoma (AT17) have given direct insight into the number and clonal expansion of stem cells in situ, strongly supporting the hierarchical concept. The implications are important and concern the relevance of predictive assays, possible mechanisms of accelerated repopulation, or the role of adjuvant treatment strategies.

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Use of the term ‘stem cells’ in conjunction with tumour cell populations has been a matter of controversial discussion (1, 2). Nobody will deny that tumours contain cells with infinite reproductive ability, but many investigators will doubt that this justifies the term and its conceptual consequences. The term derives from the organization of normal lineages and is particularly well exemplified by the expansion of bone marrow stem cells in the spleen of lethally irradiated mice, where a single founder stem cell can rapidly multiply and grow into a nodule of about one million cells. Most of these cells are partly differentiated, along either the erythropoietic or the granulocytic pathway, but there is also a large proportion in each colony of rapidly proliferating transit cells. At day 10 the absolute number of stem cells increases from 1 to 10000, but their relative number drops from 100% to 0.1%, i.e. the same proportion as met under steady-state conditions in bone marrow. Temporarily, therefore, the stem cells also expand, but increasingly the output from stem cell divisions must be directed down the differentiation pathway.

Normal stem cells hence display a number of characteristic properties (see Table 1, modified after (3)). They constitute a small subpopulation capable of self-maintenance and at the same time provide the necessary supply for normal cell turnover and tissue integrity under normal conditions by a steady input of amplifying cells with

limited proliferative capability; they are also capable of regenerating and rescuing tissue after insult, and in order to do so they must be able to up- and down-regulate the balance between self-replication and cell input into the amplification compartment. Unequivocal markers are usually not available, but sometimes stem cells may be enriched by means of their surface properties or location.

The question to be discussed is which of these features are we willing to sacrifice when adopting the term ‘stem cells’ also for tumours. Certainly, cells do exist in the tumour that self-replicate and in fact over-replicate. Also, they can rescue the tissue, as we know from local recurrence after radiotherapy, demonstrating the ability of producing unlimited progeny. Even pluripotency sometimes exists, e.g. in teratomas and some adenocarcinomas of mixed histology. To justify the term ‘stem cell’, however, we need evidence to show that a substantial proportion of viable and even proliferating cells exist in the population that indeed lack these properties. If the evidence cannot be provided, then infinite proliferative ability must be a feature of the malignant phenotype per se. By contrast, if it can convincingly be demonstrated that only a minority of the viable cells have this ability, then all other cells must be their progeny and depend for their very existence on continual supply for renewal and replacement.

Table 1*Properties of stem and non-stem cells in a cell lineage*

	Stem cells	Transit cells	End cells
Small subpopulation	Yes	(No)	No
Capable of self-maintenance	Yes	No	No
Pluripotency	(Yes)	No	No
Capable of tissue regeneration	Yes	Limited	No
Capable of large progeny	Yes	Limited	No
Flexibility (over-replication)	Yes	Yes	No
Markers	No	(Yes)	Yes

The stemness or unlimited proliferative capacity of the cells in question is tested by their ability to form clonal colonies in vitro and in vivo (4). While the criteria are clear, it is understood that the test procedure itself creates an artificial situation. All assays designed to reveal clonogenicity require severe disruption of the tumour tissue. The same argument holds, of course, if stem cells are diluted in situ by a high radiation dose, which inevitably incurs damage also to the environment of the malignant target cell. All test procedures therefore may interfere with clonogenic performance.

TRANSPLANTATION ASSAYS

The most straightforward attempt to measure cell clonogenicity seems to be in vitro plating, but a great many biological arguments can be raised that cultivation of dissociated cells in agar and medium constitutes an artificial environment. Therefore, at least for tumours that are not adapted to growth in vitro, transplantability studies are considered to be more meaningful. In 1958, the first experiment of serial dilution was undertaken by Hewitt (5), to measure the dependence of successful tumour grafting on the number of cells inoculated. The TD50 of the mouse leukaemia tested in this pioneer experiment turned out to be about 4 cells, a low number consistent with a high stemness of the tested population; beyond that, the dependence of take rate on cell number agreed well with the curve shape predicted by Poisson statistics if a positive take depended on the presence of at least one or more functional cells. So the straightforward interpretation was that 1 out of 4 cells was a stem cell and the presence of one such cell was sufficient for leukaemia development, while 3 out of 4 cells were not stem cells. Single-cell kinetics were subsequently observed for a great variety of tumour systems and overall may apply for half of the systems tested. By contrast, the TD50 values showed enormous variability between 1 and 17000 in the isogenic spontaneous tumours investigated by Hewitt et al. (6). If these data are extended by a similarly comprehensive study to be described below (7), the published TD50 values would range from 1 to > 100000 (Fig. 1).

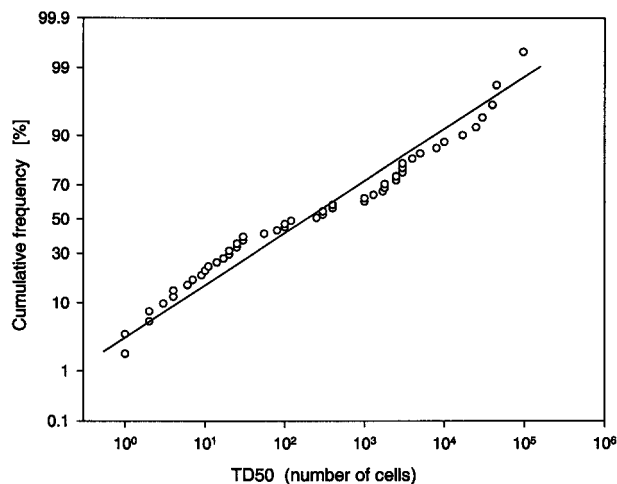


Fig. 1. Cumulative distribution of TD50 values determined with rodent tumour systems (from (18)).

Remarkably, assessment of transplantability has also been attempted in patients. One quantitative study on autologous transplantation of human tumours by Southam et al. (8, 9) includes 59 patients with advanced ovarian, gastrointestinal and epidermoid cancer, and sarcomas. Mechanically prepared suspensions of 10^5 to 10^8 cells were injected, frequently with additional trokar implantation of fragments. The overall take rate was 14/59, with most of the positive grafts verified histologically. Despite some deficiencies, such as an incomplete observation period, the crude estimate suggests that the TD50 across tumour types must have been greater than 10^5 cells. Apparently, there were also a number of earlier anecdotal reports on autologous transplantation, which also amounted to very low success rates (quoted in (9)).

The results obtained by quantitative transplantation have been explained in different ways. The mechanical and enzymatic stress imposed by dispersing tumour tissue into a single-cell suspension is certainly a substantial stress factor. Also, the transplanted cells lose their previous intercellular contacts along with, for example, paracrine signals, and are put into a new microenvironment that is certainly different from the old one. These two aspects have been dubbed the seed and the soil factor (1). Indeed, in the human study the interpretation was that all malignant cells had the potential to graft but were overruled by 'inimical host factors' that awaited further characterization (9). Such factors could conceivably be immunological host responses, which are known to be associated with high TD50 values, but in their studies Hewitt et al. (6) convincingly excluded this factor for tumours of spontaneous origin. Still, even in isogenic systems, local 'soil' factors do play a role, as is suggested by the fact that local (but not distant) injection of a large number of lethally irradiated tumour cells along with the viable inoculum can reduce the TD50 in many tumours (10), although rarely by

more than a factor of 10. The supposed mechanism entails the trapping of viable cells that would otherwise be lost. Taking the vast experience of transplantation experiments collectively, however, it is unlikely that the technical and biological factors involved in the assay can explain the vast but reproducible range in TD50 values. This lends support to the alternative explanation that high TD50 values reflect confined or fairly small stem cell fractions in these tumour cell populations. By contrast, the well-used anaplastic and highly passaged rodent tumour systems, often also adapted to growth *in vitro*, may be highly enriched in the proportion of clonogens, akin to established cell lines.

Even results from well-controlled experiments thus leave some uncertainty about how reliable absolute TD50 values may be. It seems straightforward enough to calculate a numerical stem cell fraction from TD50 assays and *in vitro* plating data, but in fact this may be a conceptual simplification. If one considers that these assays test the individual cells for their clonogenicity or stemness, the probabilistic nature of stem cell performance must be taken into account (3, 4, 11). That is to say, a given stem cell has a finite chance of becoming extinct simply because it can give rise to two non-stem daughters. Whether this spontaneous extinction probability is reproduced or altered when the cell is put into the assay environment is entirely unknown. The addition of lethally irradiated feeder cells might have a promoting effect in this regard, beyond the more mechanical trapping effect, by acting towards lower extinction probability. This provides an analogy to the effect of feeder cells *in vitro*, which certainly do not act through spatial trapping.

COMPARISON OF ENDPOINTS

From the above arguments it seems possible and even likely that there is no such thing as an absolute and 'true' figure quantifying the stem cell fraction in a tumour. What is measured is more likely an effective stem cell fraction, which must be taken as an operational figure under the circumstances of the test procedure. An important aspect therefore is to investigate how results from different assays agree in their quantitative estimates of stem cells, either absolutely or at least in terms of a correlation.

Hill and Rauth (quoted in 7) compiled such data from a panel of 12 spontaneous mouse mammary carcinomas, contrasting the clonogenicity in agar and the TD50 derived from quantitative transplantation. The relationship was well described by a regression line with slope-1, but the intercept indicated that clonogenicity in agar was nearly 10 times higher than predicted by the transplantation assay. This is an interesting result, considering that CFEs of human tumours with current techniques are 0.1% or lower (gross average across tumour types), while in analogy, the historical autologous transplantation experiments suggested higher TD50 values. In a subsequent joint study of

the Ontario Tumor Center and the MD Anderson Cancer Center, the TD50 values and TCD50 values (following single-dose irradiation) were compared in 25 tumour systems that were mostly in first or early passage (7). For the subset of mammary tumours investigated at the Ontario Tumor Center (treated with clamp doses) the TD50 values ranged from < 10 to $> 10^5$, and displayed a well-defined negative correlation with the TCD50 dose. The slope of this correlation translates into a D_0 of 3.9 Gy for the anoxic tumour stem cells.

Despite some scatter in these data, the study demonstrates that murine tumours differ considerably in their stem cell content, suggesting low or very low effective stem cell fractions in a large proportion of tumours. Yet, the authors also emphasize that they have shown a correlation rather than determined absolute stem cell fractions, again because of the uncertainty about how much the apparent results of TD50 and TCD50 reflect 'true' values or superimposed assay-specific environmental conditions. These arguments are difficult to refute. As long as measurements of stem cell numbers require the technique of excision assays, the relevance of such figures for the *in situ* situation of irradiated tumours remains doubtful.

Experimental and host factors that could conceivably impair the expression of stem cell potential and thereby cause high TD50 values are listed above. Similar arguments have been raised to explain the discrepancy between the number of viable tumour cells in macroscopic rodent or human tumours and their radiocurability (1, 2, 12, 13). If not explained by small stem cell fractions, radiation effects beyond the stochastic dose-dependent cell inactivation have to be invoked, such as the bystander effect, heritable radiation damage, or the massive tissue necrosis and vascular breakdown seen after curative doses. Yet there is no sound experimental evidence to support this speculative interpretation (14).

In summary, then, results from a large proportion of tumours tested by various functional assays strongly suggest that stemness is not a property of *all* malignant cells. Depending on tumour type, the effective stem cell fraction appears to be small compared with the number of morphologically viable cells and even compared with the number of rapidly proliferating cells that must be assumed to be nutritionally well supported.

STEM CELLS AND POPULATION STRUCTURE

One interpretation proposed to explain *apparently* low stem cell fractions was that of a 'classless' population of tumour cells. This model does not in fact imply any structure, because it assumes that all cells express a rather low clonogenicity potential (2). When tested by transplantability or therapeutic insult, the response would indeed be the same as that expected from a small fraction of

'fully capable' stem cells. Limited clonogenicity, however, is something that is intrinsic in the concept of a stem cell unless one assumes a perfectly deterministic division pattern where at least one daughter cell retains stemness. By contrast, a stochastic pattern implies a finite chance of an individual cell to produce two non-stem daughters and thus to become extinct. If all cells are alike in this respect, the population as a whole (comprising e.g. 10^9 cells) will still proliferate and almost certainly not become extinct. Yet, the rate at which it would grow would be extremely slow. This follows from the dependence of extinction probability (ω) on the probability (p) of producing two stem cell daughters in a division, given as $\omega = (1 - p)/p$. The chance to carry on through a next division for the individual cell is thus very small, with the consequence that growth becomes extremely slow, and incompatible with the growth rate observed in mouse tumours.

The Canadian Stem Cell School has proposed an alternative interpretation. In an attempt to accommodate the data on tumour stem cell measurements in a comprehensive concept, several authors have presented a model based on the assumption that malignancies (frequently well-differentiated carcinomas in man) are 'caricatures' of their parental tissues and that this also included their proliferative structure (15, 16). The proliferative organization of the tumour is assumed to be a dynamic compartment system that is hierarchically structured. Stem cells at the top of the pyramid give rise to both stem cells and non-stem cells, and the latter enter a defined transit compartment with limited proliferative capacity finally to become end cells subject to cell loss. While in steady-state populations the stem cell number remains constant, the hallmark of malignant populations is a fixed over-replication rate of stem cells. This is mathematically expressed as the probability with which two stem cell daughters are generated in the average stem cell division. In normal steady state this probability or partitioning factor is 0.5, but may change under various physiological and pathological conditions; in the malignant state the factor is permanently and irreversibly set at > 0.5 . The model describes how the proportions of compartment size depend on p and the number of transit generations or the loss rate of end cells, and particularly addresses the evolution of compartments in the process of clonal expansion from an individual stem cell. The pivotal role is played by the partitioning factor p , and as all compartments downstream depend quantitatively on the input from the stem cell compartment, the growth rate of the entire population necessarily proceeds at the growth rate of the stem cell population itself (15). As over τ generations (cell cycles) the number of stem cells increases from $S(0)$ to $S(t) = S(0) \cdot (2p)^\tau$, it appears that for a p of 0.51 (close to steady state), no less than 20 cell cycles are needed to double the stem cell number. Although in the model the compartment dynamics are treated in a rather deterministic way, it must not be overlooked that the

individual stem cell division is still probabilistic and subject to the risk of extinction as defined above.

PERFORMANCE OF SURVIVING STEM CELLS IN THE IRRADIATED TUMOUR

Some years ago we isolated a mammary carcinoma (denoted carcinoma AT17) from our C3H mouse colony that avoids some of the methodical problems mentioned above in that it facilitates the intratumoural quantitation of surviving stem cells (17, 18). The reason for this resides in a peculiar histological pattern of regression and regeneration after radiation, as depicted in Fig. 2. The untreated tumour shows a nodular parenchymal morphology, with active proliferation in the peripheral layers of these structures, but at all times a few older nodules are also seen that apparently undergo spontaneous involution. Following radiation doses greater than 30 Gy (clamp), this nodular structure breaks down and the dead cells clear away within a week, leaving behind a rather acellular matrix. Also around this time, regeneration is visibly resumed, showing up as small cell buds which, until day 18, grow into discrete epithelial spheroids of several hundred or thousand cells. The remarkable features of this spatial and temporal pattern are that (i) the dead cells clear away rapidly without excessive tumour shrinkage; (ii) the surviving clonogens regrow into individual colonies that stay well demarcated; and (iii) this process of clonal expansion occurs in a synchronous wave, resulting in a stage of mature compact colonies in a narrow time window. Surprisingly, this stage does not lead on to gross recurrence. Instead, all colonies undergo a synchronous wave of involution in which degeneration begins in the centre and progresses radially outwards, until only a narrow cellular rim is left. It is only after a considerable dose-dependent lagtime that a secondary outgrowth occurs from the rims and eventually restores the tumour. From the dose levels used, it is clear that the majority of, if not all, clones must arise from single surviving cells; also, the founding cells must be truly clonogenic as the median progeny in a clone is > 1000 cells. Finally, it appears that the secondary degeneration is programmed in the natural growth history of the original nodules and the synchrony is simply induced by the synchronous regrowth after irradiation.

The spheroidal clones thus represent survival of clonogens. With increasing doses, a gradual dilution of surviving cells becomes visible that ultimately leads to an increasing proportion of tumours with no single surviving cell (Fig. 3). By scoring serial histological sections this dependence was quantified both for single and fractionated irradiation (Fig. 4). Although the response to dose levels smaller than 32 Gy cannot be measured directly because of coalescence, there are enough data to facilitate a statistically meaningful back-extrapolation to dose zero. The number of clonogens at risk in a 100 mg tumour before irradiation

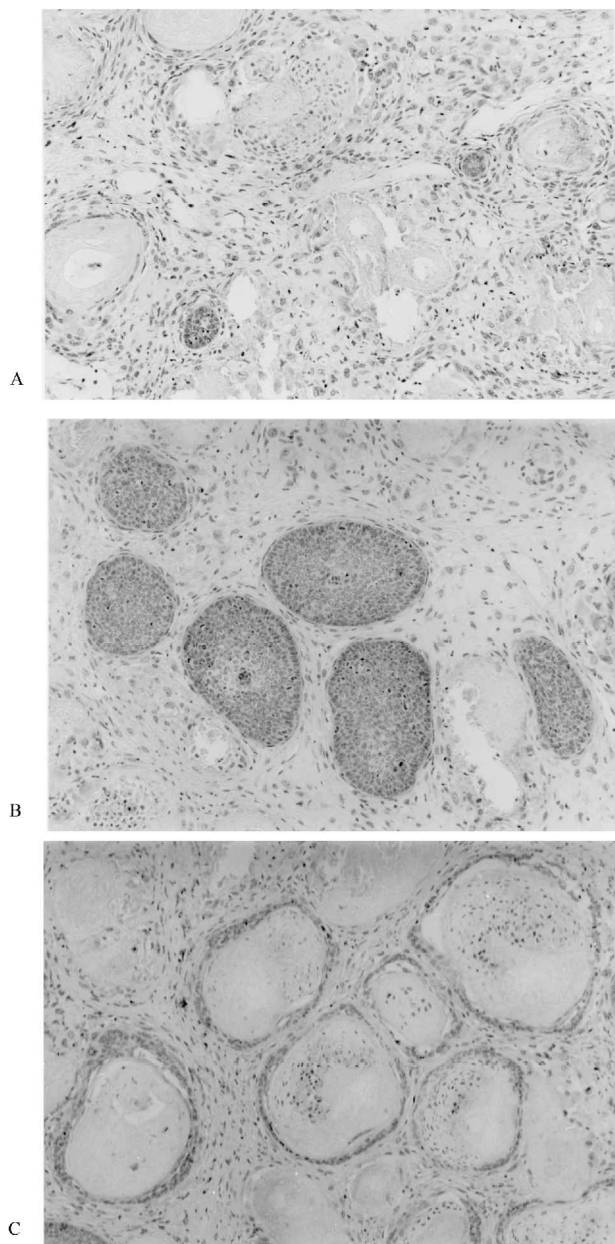


Fig. 2. Histological changes and intratumoral clone formation in carcinoma AT17 after irradiation (32 Gy, clamp). A: Early cell depletion and incipient clonal regeneration, 8-day post-irradiation. B: Stage of compact clone formation, reached 18-day post-irradiation. C: Synchronous involution of clones, 28-day post-irradiation. $\times 90$.

estimated by this extrapolation is about 2×10^6 . This corresponds to about 5% of all tumour cells counted by morphometrical methods, or to about 10% of the proliferating cells (growth fraction) in this tumour.

In view of the impressive temporary involution that actually does not spare a single clone, the question that must be asked is how many initial clones will eventually recover to repopulate the tumour. In a large experiment involving single-dose irradiation under ambient and anoxic

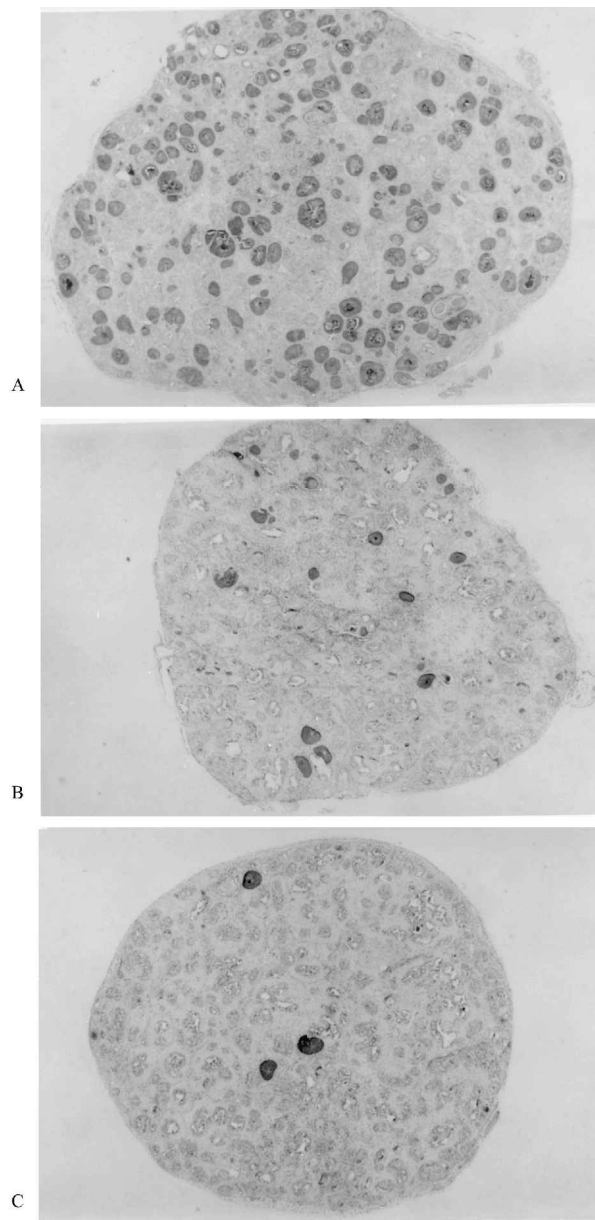


Fig. 3. Cross-sections through AT17 tumours 18 days after single-dose irradiation (clamp) with 38 Gy (A), 43 Gy (B) or 50 Gy (C), demonstrating dilution of clone number with increasing dose. $\times 10$.

conditions, clone numbers were scored 2.5 weeks after irradiation while permanent local tumour control rates were assessed after 18 months. The top panel of Fig. 5 shows clone number per individual tumour, with tumours devoid of clones indicated at the abscissa. There is an amazing variability in clone number over the whole dose range, yet with increasing dose the frequency of zero clones increases, both to ambient and clamp irradiation. In the bottom panel of Fig. 5 the plotted curves represent the cure probability as calculated from the actuarial local control data, whereas the data points shown reproduce the

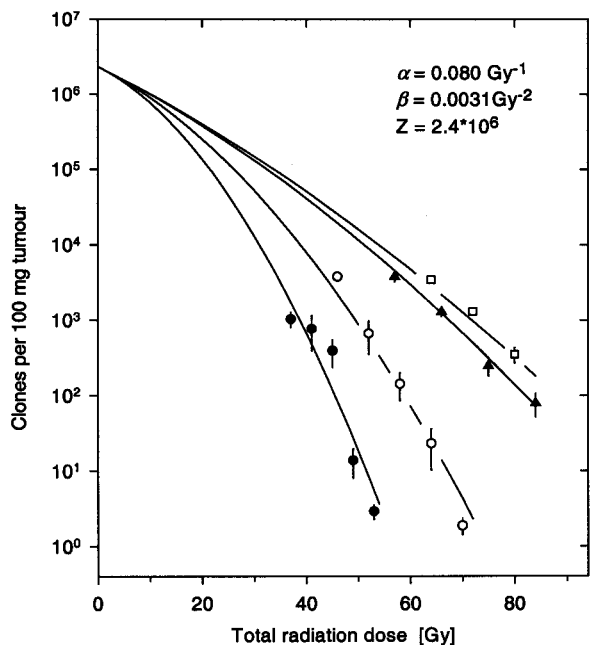


Fig. 4. Dose dependence of clone frequency on fractionated irradiation under clamp conditions. Symbols denote 1 (●), 2 (○), 6 (▲) and 8 (□) fractions. The estimated linear-quadratic parameters and number of clonogens (Z) are given in the insert (modified from (18)).

proportion of zero-clone tumours taken from the top panel. There is a general trend that zero-clone tumours underestimate the clinical cure probability, which in turn indicates that each clone encountered at day 18 has a probability of less than 1.0 to persist and eventually repopulate the tumour. However, a binomial correction that reduces the persistence probability from 1.0 to 0.5 or 0.25 is sufficient to reconcile the data, indicating that 1 out of 2 or 3 clones will succeed in the long run.

FUNCTIONAL TESTING OF CLONAL EXPANSION

Another important question concerns the proliferative structure of the cell population that constitutes a clone. The founding cell of each individual clone is a stem cell, which over 9 to 12 effective doublings expands into a numerous progeny of cells that up to the incipient stage of involution are morphologically quite indistinct. The question here is whether the founding stem cell over this period gives rise mostly to stem cells or whether, along with clonal expansion, a population structure has evolved similar to that in the undisturbed tumour.

The answer requires the assessment of stem cell numbers per clone, a task attainable only by a functional assay. Several experiments were carried out to this end, using a retreatment design with a priming dose, D1, administered to initiate clones, and graded secondary doses, D2, given later at defined stages of clone development.

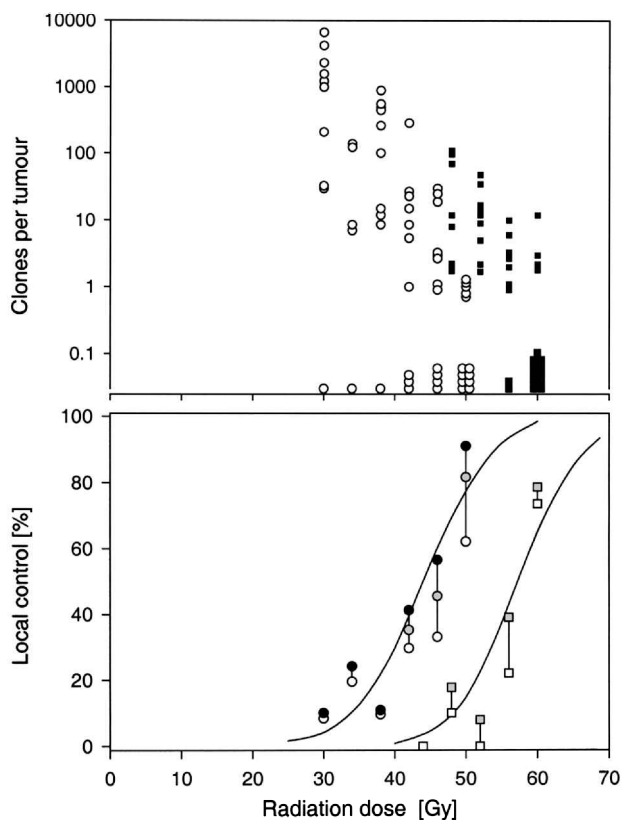


Fig. 5. Dose response of AT17 tumours to single-dose irradiation under ambient (circles) or anoxic (squares) conditions, comparing intratumoral clone formation and clinical tumour cure. Top panel: Clone numbers measured 18 days post-irradiation. Tumours with zero clones are listed above the abscissa. Bottom panel: Local tumour control as calculated from the actuarial cure data 18 months post-irradiation (curves) or as predicted by the frequency of zero-clone tumours shown in the top panel. Open symbols denote the observed frequency, grey and black symbols are the frequencies resulting when the probability of the individual clone to persist is assumed to be only 0.5 or 0.25, respectively (modified from (18)).

Previous experiments had indicated that clones in which one or more stem cells survived a secondary test dose were indeed able to regrow to the same pattern of compact clones within the usual assay time of 18 days.

The results shown in Fig. 6 demonstrate a marked horizontal shift (or $D_{0.1}$) towards higher test doses for the 10-day interval, and even more so for the 14-day interval. This shift reflects the multitarget 'threshold dose' caused by a growing number of stem cells per clone. On the basis of previous information on cellular radiosensitivity, the experimental dose increments can be converted to about 5 and 6.5 stem cell doublings afforded within 10 and 14 days, respectively. A similar increase in stem cell number is visible from a back extrapolation of the fitted curve (B), which indicates that a total of 10^4 cells at risk are contained in about 10^2 clones. Interestingly, the 18-

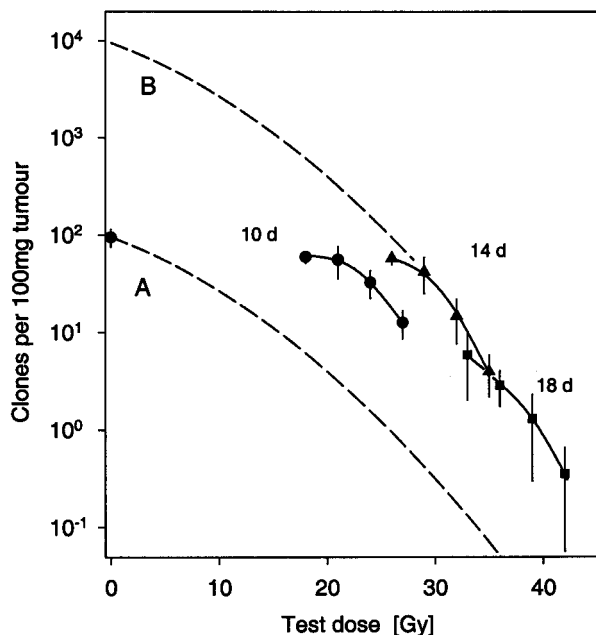


Fig. 6. Results of a retreatment experiment to measure the stem cell number in clones at various stages of regrowth. Following a priming dose of 38 Gy, graded test doses were delivered after 10, 14 or 18 days and tumours excised 18 days later. The curves describe the theoretical response of single cells (A) and the response of the 14 and 18 day clones (B) and have been constructed using background information (see Fig. 4).

day data indicate no further growth in stem cell number, although the total cell number per clone from day 14 to 18 still rises from 800 to about 2000, as assessed by morphometry. This suggests that the impending stage of differentiation and involution is preceded by a check in stem cell multiplication.

The data presented thus demonstrate a declining rate of stem cell expansion terminating in a complete check some days before the clones enter the visible involution phase, suggesting a programmed sequence of events. As involution proceeds, cell death spreads from the clone centre to the periphery in an ordered fashion and eventually leaves very few marginal cells. Provided the stem cells were distributed equally through the clone, the physical loss of such a large proportion of cells (>95%) would be expected to have a detectable impact on radioresistance. In a search for such fluctuations, retreatment experiments were extended to cover intervals from 18 to 50 days, i.e. the entire period from the mature compact stage to the phase of secondary outgrowth. Preferably, we would have used the same assay as above for these measurements, but once clones have entered the involution phase (i.e. 18 days or more after D1) their response to test doses becomes histologically irregular and the scoring of regenerating clones impossible.

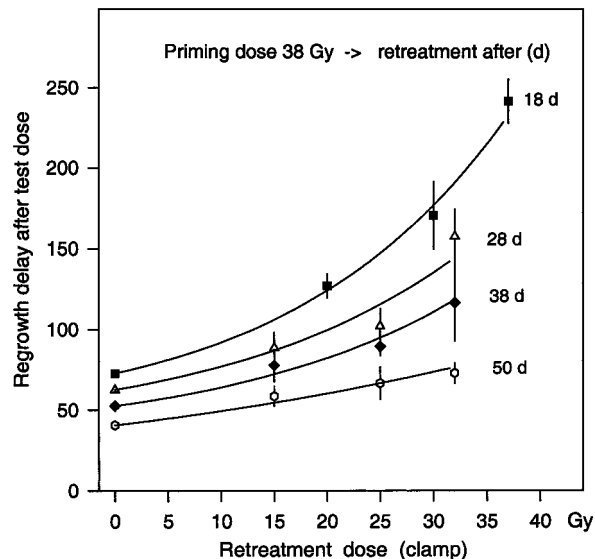


Fig. 7. In situ tumour response (regrowth delay to twice treatment size) to a protocol as shown in Fig. 6, but with longer retreatment intervals (indicated at the curves). The dose responses indicate a continuous increase in radioresistance, despite the massive cell loss during clone involution (days 18 to 28).

The presence of a fluctuating number of stem cells was therefore tested by a tumour regrowth delay assay. The dose-response curves to test doses (clamp) delivered 18, 28, 38 and 50 days after the priming dose are presented in Fig. 7. There is a steady decrease in delay, with no hint of a temporary rise after day 18, even when the curves are not normalized to the day of the test dose. Thus tumours become increasingly resistant over the period from day 18 to 28, when in fact the vast majority of viable epithelial cells are lost from the clones. The responses are best explained by an arrangement of the stem cells on the marginal cell layer(s) of each clone. One hundred stem cells that selectively survive clone involution would cover as little as 0.1 to 1% of the clone surface and hence be microscopically undetectable. The gradual increase in radioresistance beyond day 18 may reflect slow stem cell repopulation setting in after the temporary check, but is difficult to quantify from the dose response shown in Fig. 7.

In summary, the clonal expansion, subsequent involution and eventual growth into a recurrence from a single surviving stem cell can be described by changes from an initially high partitioning factor (p) to a value of ≤ 0.5 (on day 18), with an ensuing recovery to > 0.5 . This is similar to what occurs during regeneration of CFU-S in the irradiated bone marrow and possibly indicates a basic feature of clonal regeneration. This may reflect an intrinsic stem cell property or, more likely, may be the result of cellular interaction where demand is regulated by cell contacts or paracrine cytokine signals.

CONSEQUENCES AND TRANSLATIONAL ASPECTS

The overriding evidence from a variety of functional assays supports the view that in all but artificially anaplastic tumour systems only a fraction of the malignant cells are endowed with infinite proliferative capacity. They generate stem and non-stem daughter cells in a probabilistic fashion, which introduces a stochastic risk of extinction for the individual stem cell, but for the entire population is typically associated with continuous over-replication and growth. The majority of viable proliferating cells have only limited proliferative ability, justifying the concept of a hierarchical structure of the malignant population in analogy to normal lineages. The most important single factor is the stem cell partitioning factor (p) that also determines the growth rate of the entire population.

In many ways this concept of a hierarchical population structure in differentiated carcinomas must impact on biological and therapeutic considerations. Stem cells are the prime targets for tumour eradication by radiotherapy and a reasonable estimate of their effective number is indispensable to validate our quantitative understanding of dose-cure relationships. This is also true if we consider further adjuvant mechanisms, such as tumour-specific immunity, bystander or other non-targeted radiation effects, all of which can only reasonably be assessed for their effective contribution on such a basis.

We do not have the means directly to identify stem cells or to monitor them during a course of treatment. This makes the many approaches aimed to derive prognostic factors by testing the abundant tumour cell questionable or at least crudely empirical. This may even apply for so-called 'clonogenic' assays that may often test transit cells (19). Yet, as pointed out more recently, transit cells may well share with their parental stem cells relevant characteristics such as the responsivity to the antiproliferative effect of radiation or drugs and features of drug metabolism (18).

Most importantly, the generation of stem and non-stem cells as governed by the partitioning factor p must be regarded as a flexible process. This is another analogy to normal tissues, where from steady state ($p = 0.5$) to totally symmetrical stem cell divisions ($p = 1$), the production of stem cells can be regulated over a wide range in response to demand. The extent of accelerated repopulation in squamous cell carcinomas, measured indirectly by the increase in resistance associated with overall treatment time, can only be explained if a similar flexibility in stem cell production, or increase in p , is assumed to occur. The molecular key factor(s) or signalling cascades that ultimately control division symmetry, and hence stem cell production, are as yet unknown. Their identification would be a crucial step towards effective control of accelerated repopulation in particular, or even malignant growth in general.

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