**RADIOGENIC NEOPLASIA IN
THYROID AND MAMMARY CLONOGENS**

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Background

The induction of cancer by ionizing radiation is a matter of great practical importance to the nuclear industry, to national defense, to radiological medicine and to the general public. It is increasingly apparent that carcinogenesis is a leading dose-limiting effect of radiation exposure. The thyroid and mammary glands are among the most sensitive human tissues to radiogenic initiation of cancer, and there is a profoundly higher risk of neoplastic initiation in these glands among individuals irradiated before or during puberty than among those exposed in later life (NAS90; La93, 94; Th94; Ro95).

We developed unique quantitative experimental models to investigate and characterize the cells of origin of thyroid and mammary cancers and the effects of radiation on them (Cl85). To study these progenitor cells *in vivo* it is necessary to have a system by which their concentrations, total numbers and responses to radiation and other factors can be measured. It is a truism that not all cells in a tissue are equally sensitive to neoplastic initiation. We reasoned that the progenitor cells are most likely members of that subpopulation that is necessary to maintenance of normal tissue cell numbers and to repair and replacement after tissue damage. We further reasoned that such cells would likely be responsive to specific mitogenic stimulation by hormones. On the basis of these considerations, we developed quantitative rat thyroid and mammary epithelial cell transplantation systems. In these systems, the graft recipients are "conditioned", *i.e.* are hormonally manipulated to stimulate proliferation of the division-competent cells. In the thyroid system, recipient rats are conditioned by elevation of their TSH (thyroid stimulating hormone) titers by thyroidectomy and feeding of an iodine-deficient diet. In the mammary system, recipient rats are conditioned by elevation of their Prl (prolactin) titers by transplantation of a prolactin-secreting MtT (mammatropic pituitary tumors) and/or by other methods described below. Serial dilutions of monodispersed cells of either gland are inoculated into gland-free fat pads of hormonally manipulated recipients. Three or four weeks later, whole mounts of the graft sites are stained and examined for the presence of one or more glandular structure(s). The assay endpoints are the presence or absence of thyroid or mammary structures in the graft sites. When monodispersed thyroid epithelial cells are transplanted to appropriately conditioned recipients, about one of one hundred of the grafted cells will proliferate and differentiate to give rise to a multicellular FU (follicular unit) comprised of one or a few multicellular functional follicles (Cl78; De79). If mammary cells are transplanted, and the recipient rats are conditioned by hyperprolactinemia alone, depending on the cell inoculum preparation method, about one of ~300 grafted cells will give rise to an AU (alveolar unit). An AU is generally comprised of a spherical, freestanding structure lined with secretory cells and filled with secretion

(Go77). If the mammary cell recipients are glucocorticoid-deficient as well as hyperprolactinemic, structures will be a DU (ductal unit) comprised of branching ducts and endbuds with scant secretion (Ka91). The evidence indicates that FU, AU and DU are monoclonal in origin; hence, the cells from which they arise have been termed *clonogens* (CI85; CI90).

The fraction of graft sites with one or more thyroid FU or mammary AU or DU are then used to estimate the fraction of cells inoculated that were competent to yield a glandular structure when grafted and hormonally stimulated according to the transplantation formula of Porter *et al.* (Po73):

$$P = 1 - \exp (-M)$$

and:

$$M = \log K + S \log Z$$

where P is the probability that an FU, AU or DU will develop in a given graft site, M is the mean number of clonogens grafted per site in that concentration of transplant inoculum, K is the clonogenic fraction, Z is the mean total number of cells per graft site of inoculum concentration, and S is the slope of the relationship (CI85). S and K are estimated with the aid of a maximum likelihood iterative procedure. According to the model of Porter *et al.*, if the glandular structures scored are monoclonal in origin and there are no cell interactions during their formation, S will approximate 1.0. If two cells are required to form a structure, the S will approximate 0.5, and if more cells are required, S is progressively smaller (Po73; Go77). In the case of mammary cell assays, S virtually never deviates significantly from 1.0, falling in the range of 0.9-1.1. Thyroid cell assays usually yield S values in the range of 0.75-1.05; suggesting probable hormonally based interactions of the grafted cells with the host (CI90). The clonogenic fractions (K values) obtained from repeated assays of normal cells prepared in the same fashion and similarly grafted in comparable recipient rats are very reproducible. The fractions of clonogens surviving a given treatment, say 5 Gy X-rays, can then be readily calculated according to the formula:

$$F = K_C / K_R$$

Where F is the fraction of clonogens surviving 5 Gy, K_C is the clonogenic fraction of unirradiated control cells, and K_R is the clonogenic fraction after 5 Gy irradiation. The assay had been used for the first direct studies of radiation dose-thyroid or mammary clonogen survival relationships after irradiation *in vivo*, to describe repair of potentially lethal damage in clonogens irradiated *in vivo*, and most importantly to describe radiation initiation of cancer on a per clonogen basis (CI89, CI90). In the latter case, when irradiated mammary or thyroid cells are transplanted as above, and the recipient rats are maintained for long term observation, cancers arise in many of the graft sites within the

clonal structures. Hence the cancers are clonogenic in origin, and the number of cancers observed per grafted clonogen in grafts of small clonogen numbers is a conservative estimate of the frequency of radiogenic neoplastic initiation per cancer-susceptible cell (Mu84; Cl86).

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The first large study of carcinogenesis in grafts of known numbers of surviving 5 Gy-irradiated thyroid clonogens led to two major observations: 1) radiogenic initiation of thyroid cancer is a highly frequent event at the cancer-susceptible cell level, and 2) grafted cell number-dependent interactions suppress promotion-progression of initiated thyroid clonogens to cancer (Mu84). The first of these observations led to the conclusion that in this system, radiogenic initiation occurs at orders of magnitude greater frequency than any known radiogenic mutation in a specific locus.

We designed an experiment to observe whether the suppression of promotion-progression of initiated cells seen in grafts of large irradiated thyroid cell numbers could be mimicked by addition of unirradiated thyroid cells to the graft inocula of irradiated cells (Wa88). The results of this experiment show the latter to be the case; the addition of unirradiated cells to the irradiated inocula suppressed tumor formation in every experimental group tested (Fig. 1). Furthermore, the addition of unirradiated thyroid cells also suppressed histopathologically observed vascular invasion. Serum TSH levels were measured forty-four weeks after grafting of either five or 180 irradiated or unirradiated thyroid clonogens. All the recipient rats were thyroidectomized; half the rat groups were fed an iodine deficient diet and the remainder normal rat chow. In all comparison groups, the rats fed iodine deficient diet and had received five grafted clonogens each had four- to ten-fold lower TSH titers than rats on the same diet that had been grafted with 180 clonogens; in the groups fed normal chow, TSH levels were 23- to 25-fold lower in the 180 clonogen-grafted than the five clonogen-grafted rats (Wa88). Thus the suppression of promotion-progression is likely due, at least in part, to feed-back inhibition of hypophyseal TSH release by the larger thyroid hormone levels secreted by the follicles formed by clonogens in the grafts of larger cell numbers. The secretion of thyroid hormones by the grafts was reduced but not abolished in the groups fed iodine deficient diet (<50 ng I/g diet) (Wa88). There remained the possibility of cell number-dependent effect mediated by local cell interactions, as seen in some comparable systems (see Ka95).

A subsequent detailed study of the restoration of thyroid function in thyroidectomized rats by transplantation of monodispersed cells demonstrated the dependence of the restoration on the numbers of cells transplanted as well as upon the

iodine content of the diet (Do90). At grafted thyroid cell doses $\leq 1.2 \times 10^4$ per rat, T4 (thyroxin), T3 (triiodothyronine) and TSH did not reach the normal ranges during seven weeks after transplantation, whereas all three hormones did so at the two larger cell doses in rats fed normal rat chow (Fig.2). Surprisingly, in rats fed the iodine deficient diet, although T4 levels remained at or near total thyroidectomy levels in rats receiving all of the grafted thyroid cell numbers, T3 rose to approach the normal range in rats given the two highest cell numbers, and TSH titers fell toward, but did not reach, normal levels after the highest cell dose (Fig.2). There was an inverse relationship between grafted cell number and radioiodide uptake by the grafts eight weeks after thyroid cell transplantation (Table 1). These results demonstrate that reestablishment of the hypothalamic-hypophyseal-thyroid feedback system by the FU that developed in the graft sites was quantitatively related to the numbers of grafted thyroid cells (Do90).

It had been noted that 7 Gy low LET radiation given to mammary cells one day before they were grafted in recipient rats manipulated to induce hyperprolactinemia and glucocorticoid deficiency produced fewer mammary cancers than the same radiation dose delivered 35 days after transplantation to the sites of grafting of the same number of mammary clonogens. It was concluded that this effect might be due to a hormonally stimulated increase in the number of clonogens present at the time of irradiation 35 days after grafting, and perhaps also to a higher proliferative rate in the grafted clonogens at the time of exposure. We postulated that it might be possible to develop a hypersensitive rat model for the study of radiation carcinogenesis *in vivo* by inducing an increase in the concentration of mammary clonogens before irradiation. Prl+ (hyperprolactinemia) was induced by intrasplenic implantation of a pituitary gland adjacent to a Silastic capsule containing estrone; Glc- (glucocorticoid) deficiency was induced by adrenalectomy. This manipulation results in Prl+/Glc- without peripheral hyperestrinism. After adrenalectomy, the rats are maintained on their choice of saline or tap water and, in long-term experiments, weekly injections of 2 mg. deoxycorticosterone. Forty-eight to 53 days after surgery to produce Prl+/Glc-, the following changes were noted in the inguinal fat pads which each contain three mammary glands: total DNA was increased threefold, tritiated thymidine uptake into DNA was increased over tenfold, the total mammary cells recoverable for transplantation assay were increased about sevenfold, and the total assayable clonogens were increased fourfold (Ka89).

In the carcinogenesis experiment, the day before the endocrine manipulations (day -1) one group of rats was irradiated to the whole body with 40 cGy 250 kVp X-rays, and another group with 10 cGy fission spectrum neutrons. Forty-eight days after the endocrine manipulations (day +48), three more groups were irradiated with 4, 40 or 80

cGy X-rays, respectively, and three groups with 1, 10 and 20 cGy neutrons, respectively. The animals were observed for mammary carcinoma formation. In the rats irradiated day +48, the mammary carcinoma incidence per 10^3 rat-days at risk increased linearly with X-ray dose ($R^2 = 0.998$) and with the 0.4 power of neutron dose ($R^2 = 0.995$) (Ka89) (Fig. 3). Interestingly, carcinoma incidence was not significantly higher than in unirradiated rats in the group that was exposed to 40 cGy X-rays on day -1 before the increase in both thymidine uptake and total clonogen numbers. In contrast, irradiation with 10 cGy neutrons induced about the same risk of mammary carcinomas when given on day -1 as when given on day +48. It is not certain whether the effect of treatment with Prl+/Glc- before X-irradiation is due to the increase in clonogen number, to the increase in proliferation rate at the time of exposure or both (Ka89). The lack of an observed effect of Prl+/Glc- pretreatment with the neutron irradiation, and the downward change in slope of the neutron dose-cancer effect relationship at higher doses, indicates that there likely quantitative and/or qualitative differences in the mechanisms of neoplastic initiation between low and high LET radiations.

Exposure of rats to goitrogenic agents leads to self-limited thyroid hyperplasia, goitrogenesis and TSH-responsive thyroid tumors. Some concluded that the thyroid size plateau reached following growth during chronic exposure to goitrogens was due to acquired refractoriness to mitogenic stimulation by TSH. However, late-appearing tumors suggested the alternative hypothesis that TSH-sensitive clonogens continued to be present even in plateau-phase goiters. Two goitrogenic regimens were used in tests of this hypothesis: ATA (aminotriazole) plus ND (normal diet) and potassium perchlorate ($KClO_4$) plus LID (low iodine diet). Thyroid weights plateaued at different levels during these two treatments, but serum TSH titers rose and fell similarly (Fig. 4). The clonogenic fractions of thyroid cells were determined by transplantation assay of cells from goiters from rats treated for six weeks with ATA-ND and with perchlorate-LID, and from normal thyroids from age-matched controls (Gr92). Based on these assays, three cell concentrations were chosen to contain the same numbers of clonogens for cell suspensions prepared from the age-matched controls and from the two goiter types. These cell concentrations were then transplanted to thyroidectomized recipient rats fed ND. Eight weeks later, the titers of T4, T3 and TSH in the sera the recipients were measured. The hormone data are plotted against the total numbers of grafted thyroid cells from goiters and from control glands (Fig. 5A), and against the total numbers of thyroid clonogens grafted (Fig. 5B). It is clear that the eighth week T4 and T3 titers increased and the TSH titers decreased in direct proportion to the assayable clonogen numbers grafted, regardless of origin from a normal gland or from a goiter (Gr92a). The clusters of FU formed at the graft sites of clonogens from

normal thyroids and from goiters were indistinguishable in whole mounts and on histological examination, and essentially all their cells incorporated bromodeoxycytidine during days 8-28 after grafting in thyroidectomized recipients (Gr92a).

During the rapid hyperplasia in the initial three weeks of treatment with either goitrogenic regimen, the clonogenic fractions decreased precipitously (Gr92b). When the plateau phases of goitrogenesis were reached at about six weeks of treatment, the clonogenic fraction tended toward plateau in the perchlorate-treated group, but began a rapid rise in the ATA-treated group (Fig. 6). The rise in the clonogenic fraction continued during early goiter regression after cessation of ATA administration at 12 weeks (Fig. 6). The clonogenic fraction also rose after cessation of perchlorate treatment, but did not reach normal. In the ATA-treated group, the rise in the clonogenic fraction led to an increase in total clonogens per gland to above control levels (Fig. 7). The total clonogens per gland remained below the normal glandular level in the perchlorate group, however. The difference in effect between these two goitrogenic regimens is likely related to the difference in their mechanisms of action. ATA inactivates thyroid peroxidase, an essential enzyme in the organification of iodine and iodine concentration and hence necessary to synthesis of T3 and T4. Perchlorate blocks iodide uptake at the thyrocyte membrane and so reduces T3 and T4 synthesis. Six weeks after withdrawal of the initial goitrogen treatments, when the involuted glands were again challenged with ATA-ND and perchlorate-LID, the patterns of response were similar to those following the initial challenges, but were significantly less extreme. Once again, the FU that arose in grafts of thyroid cells from plateau-phase and involuted goiters were not distinguishable from those in grafts of cells from euthyroid glands (Gr92b). Many of these data have been summarized and their implications discussed in workshops and reviews (C189; C190; C191a, b, c).

A large experiment was designed to gain further information on cell interactions including hormonal feedback during promotion-progression of radiation-initiated thyroid clonogens (Do94). Each thyroidectomized recipient rat received "test" grafts containing 90, 30, 10 and 3 unirradiated or 5 Gy-irradiated surviving thyroid clonogens in four fat pad graft sites; in addition, half of the recipient rats received a "large" graft containing 1400 unirradiated clonogens in a fifth site. All rats were fed LID (<50 ng I/ g diet) until termination. This design was chosen to chronically expose the clonogens in the "test" sites in rats without fifth "large" grafts to maximal TSH titers, and to expose all the "test" clonogens in recipient rats with fifth "large" grafts to somewhat lower TSH titers. The

"test" graft sites of irradiated clonogens in the rats without "large" grafts had higher final cancer incidences overall than did comparable graft sites in rats with "large" grafts (Fig. 8). The highest net radiogenic cancer frequencies were one cancer per ~7 and per ~22 irradiated clonogens in grafts initially containing three and ten clonogens per site, respectively. The differences in cancer incidences were most likely due to the 50% higher TSH titers in the rats without "large" grafts than in rats with such "large" grafts. The suppression of TSH levels almost certainly resulted from secretion of thyroid hormones, predominantly T3, by the FU formed in the "large" thyroid clonogen grafts despite the severe iodine deficiency (Do90). The high frequencies of cancer per surviving clonogen are conservative estimates of radiogenic cancer initiation frequencies in that generally methodological sources of inaccuracy tend to reduce rather than increase the observed frequencies as compared to the "real" frequencies. We conclude that radiogenic initiation of cancer per susceptible thyroid clonogen is a highly frequent event, and is very much more common than induced mutation frequencies at any known locus. We reconfirm that promotion of thyroid cancer from both radiogenically and spontaneously initiated thyroid clonogens occurs in relationship to TSH titers (Do94).

The risk of induction of breast cancer by ionizing radiation is greatest among baby girls and young women before and during puberty (NAS90; La93, 94; Th94). We undertook a study of the concentrations and possible changes in the radiosensitivity of rat mammary clonogens with age before, during and after puberty. We believed that a) the data might yield insight into the cellular basis for age-related susceptibility to radiation carcinogenesis, and b) the data were essential to design of experiments to directly test whether the high sensitivity of prepubertal girls to breast cancer initiation could be modeled in the rat system (Sh94). Accordingly, the total numbers of recoverable clonogens in 1, 2, 3 and 4 week-old (prepubertal), 6 week-old (pubertal), and 8 and 12 week-old (post-pubertal) female rats were determined and clonogen survival after 5 Gy ^{137}Cs γ -rays were estimated in AU-based assays. Assayable mammary clonogen concentrations were very low in one-week-old rats and rose increased exponentially until four weeks of age; thereafter their numbers tended to plateau (Fig. 9). About 10% of the clonogens survived 5 Gy in 1-4 week-old rats. Survival then increased with age and puberty at six weeks and reached ~30% survival in sexually mature eight- and twelve-week-old females (Fig. 10). Radiation dose-mammary clonogen survival curves following graded radiation doses to prepubertal two- and four-week-old rats were exponential from the origin, whereas survival curves for sexually mature eight-week-old females had a pronounced initial shoulder (Fig. 11) reflecting repair of potentially lethal damage in the older rats (Sh94).

This is the first demonstration of which we are aware of age or puberty, presumably hormone, -related differentiation of an intracellular damage repair system *in vivo*. The large carcinogenesis experiment designed in part on the basis of these experiments to determine whether the high susceptibility of prepubertal and pubertal human females can be modeled in the rat system is currently in analysis.

Finally, we are currently analyzing a large thyroid carcinogenesis experiment designed to determine whether and how a period of exposure of irradiated thyroid clonogens to the euthyroid milieu with intercellular contact within the gland might alter progression to cancer.

Closing Thoughts

The major contributions of our laboratory's efforts during the last quarter century have been:

- a) That radiogenic cancers in mammary and thyroid glands arise from stem-like clonogenic cells *in vivo*, and that these cells can be studied quantitatively *in vivo*.
- b) That radiogenic initiation of mammary and thyroid cancers is a highly frequent event at the cancer-susceptible clonogenic cell level *in vivo*, and most likely involves processes other than mutation of one or more specific genes.
- c) That promotion-progression of initiated cells to overt cancer can be suppressed by local cell-cell interactions *in vivo*.

These, and data from other groups, have led us to conclude that carcinogenesis generally involves an imbalance between epigenetic, high frequency initiation and suppression of promotion-progression of initiated clonogenic cells (Ka95, Cl96). These conclusions, now being widely confirmed by others, have important implications for future cancer research as well as for evaluation of radiation risks and protection against radiation damage.

Table 1: Effect of grafted cell number and diet on the efficiency of ^{125}I uptake by thyroid grafts originating from differing cell numbers. Table from Domann et al.

	No. of cells/ animal	n ^a	% Uptake ($\times 10^2$) ^b	Uptake ratio ^{b,c}	P ^d
Normal diet	77,500	4	3.36 $\pm$ 0.78	6.60 $\pm$ 2.55	0.08
	15,500	4	1.89 $\pm$ 0.73	3.36 $\pm$ 1.74	0.03
	3,100	4	0.72 $\pm$ 0.35	0.87 $\pm$ 0.41	0.05
	620	4	0.35 $\pm$ 0.15	0.32 $\pm$ 0.16	0.03
	Sham	4	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01	
Low iodine diet ^e	77,500	4	36.2 $\pm$ 5.2	118.9 $\pm$ 31.5	0.62
	15,500	4	34.1 $\pm$ 4.3	107.3 $\pm$ 31.7	0.005
	3,100	4	14.0 $\pm$ 7.6	25.7 $\pm$ 19.9	0.06
	620	4	2.1 $\pm$ 0.7	2.93 $\pm$ 2.41	0.17
	Sham	3	0.1 $\pm$ 0.03	0.09 $\pm$ 0.03	

^a Number of animals sampled.

^b Values shown are the mean $\pm$ SD.

^c Iodine uptake ratio = cpm per graft/cpm per ml serum.

^d Probability that observed difference in iodine uptake ratios between adjacent serial cell dilutions is due to chance (P values obtained from two-sample t tests).

^e LID2 was used for this study.

Efficiency of carcinoma development from grafted irradiated clonogens as a function of the number of irradiated clonogens per transplant site (left), or total clonogens per site (right). See Table I for numbers of unirradiated clonogens added to the indicated irradiated clonogen inocula (left abscissa) of 'mixed clonogen' grafts. Carcinoma formation from mixed irradiated-nonirradiated clonogen grafts are plotted over both abscissae.

Figure 1: from Watanabe *et al.* (Wa88)

Serum T₃, T₄, and TSH levels in thyroidectomized recipients inoculated with thyroid cells and fed a normal diet (ND). Points plotted are geometric means. Groups of animals were inoculated with the indicated cell number, sham inoculated, or left intact. Dashed horizontal lines represent the detection limits of the assays. Shaded areas represent the normal range of the specified hormone. Brackets include data points not significantly different from each other, but significantly different ($P < 0.05$) from data points in adjacent brackets at the same week. Missing data occur where fewer than two animals were sampled due to intercurrent loss or insufficient serum harvest to perform all three assays.

Serum T₃, T₄, and TSH levels in thyroidectomized recipients inoculated with thyroid cells and fed a low iodine diet (LID2). Points plotted are geometric means. Groups of animals were inoculated with the indicated cell number, sham inoculated, or left intact. Dashed horizontal lines represent the detection limits of the assays. Brackets include data points not significantly different from each other but significantly different ($P < 0.05$) from data points in adjacent brackets at the same week. Missing data occur where fewer than two animals were sampled due to intercurrent loss or insufficient serum harvest to perform all three assays.

Figure 2: from Domann *et al.* (Do90)

Effect of radiation dose and time of exposure on total mammary carcinoma risk per rat day. Solid triangle: unirradiated control group C. Open squares, X-irradiated groups 1X, 2X, and 3X exposed on Day +48. Open circles, neutron-irradiated Groups 1N, 2N, and 3N exposed on Day +48. Solid square, X-irradiated group 4X exposed on Day -1. Solid circle, neutron-irradiated group 4N exposed on Day -1. Vertical bars are standard errors. Straight line through X-ray points calculated according to $y = 3.34 + 0.05$ (cGy X rays). Curve through neutron points calculated according to $y = 3.27 + 1.14$ (cGy neutrons)^{0.4}. R^2 values are 0.998 (X rays) and 0.995 (neutrons). If the neutron dose response is assumed linear (not plotted) the relationship is $y = 3.88 + 0.17$ (cGy neutrons) and $R^2 = 0.895$.

Figure 3: from Kamiya *et al.* (Ka89)

Effect (mean $\pm$ SEM) of ATA/ND and KClO₄/LID treatment on thyroid weight (A) and TSH levels (B). ATA/ND(●); KClO₄/LID (▲); age-matched controls (■). Where not shown, error bars are contained within the symbols.

Figure 4: from Groch and Clifton (Gr92a)

A. Total grafted cell dose effect curves for T₄, T₃, and TSH synthesis (mean ± SEM) in tx - 14 recipients eight weeks after transplantation. Age-matched control cells (■); six-week ATA/ND cells (●); six-week KClO₄/LID cells (▲); age-matched intact animals (O); age-matched tx animals (□). Where not shown, error bars are contained within the symbols. B. Total grafted clonogenic cell dose effect curves for T₄, T₃, and TSH synthesis (mean ± SEM) in tx - 14 recipients eight weeks after transplantation. Age-matched control cells (■); six-week ATA/ND cells (□); six-week KClO₄/LID cells (▨); age-matched intact animals (O); age-matched tx animals (□).

Figure 5: from Groch and Clifton (Gr92a)

Clonogenic fractions ($\pm$ SEM) of enzymatically monodispersed thyroid cells during an initial goitrogenic challenge with ATA/ND (●) and KClO₄/LID (▲) (Panel A, —) and following the withdrawal of 12 weeks of treatment (Panel B, ---). (■) Age-matched controls. Arrow designates 12-week time point. The FU which arose from goiter cells in tx recipients were indistinguishable from those which arose from control cells.

Figure 6: from Groch and Clifton (Gr92b)

Total clonogenic thyroid cells per gland ($\pm$ SEM) during an initial goitrogenic challenge with ATA/ND (●) and KClO₄/LID (▲) (Panel A, —) and following the withdrawal of 12 weeks of treatment (Panel B, ----). Arrow designates 12-week time point. The estimates of total clonogenic cells were obtained by converting the thyroid DNA values to total cells (assuming 6.5×10^{-6} μ g DNA per cell) and multiplying this value by the clonogenic fraction. (■) Age-matched controls.

Figure 7: from Groch and Clifton (Gr92b)

Cancer incidence rates in grafts of irradiated and unirradiated clonogens as a function of grafted cell number and treatment group. Rate values are expressed as the number of cancers per 10^4 graft*weeks. Upper panel shows data from groups R (○) and C (●), lower panel shows data from groups RE (○) and CE (●).

Figure 8: from Domann et al. (Do94)

Total recoverable clonogens per six inguinal mammary glands of the same rats. Total clonogens were calculated from the AD_{50} values and the total recoverable cells. Vertical bars are standard errors in both.

Figure 9: from Shimada *et al.* (Sh94)

Surviving fractions of mammary clonogens that had been irradiated with 5 Gy γ rays *in situ* (calculated from data sets in Fig. 2). Vertical lines are standard errors. Note but one determination at 12 weeks.

Figure 10: from Shimada *et al.* (Sh94)

Figure 11: from Shimada *et al.* (Sh94).

Survival curves for mammary clonogens irradiated *in situ* with γ -rays when prepubertal (two weeks of age, open squares), in early puberty (four weeks of age, open diamonds) or when sexually mature (eight weeks of age, open triangles). All data points from clonogen donor rats of a given age, including those from replicate zero dose assays, were normalized by division by the mean of the zero dose groups. Solid lines were calculated according to the α/β model and fit by a computer to a second order equation. ____

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