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In silico Repopulation Model of Various Tumour Cells during Treatment Breaks in Head and Neck Cancer Radiotherapy

Loredana G. Marcu, David Marcu, Sanda M. Filip

***Abstract*—**Advanced head and neck cancers are aggressive tumours, which require aggressive treatment. Treatment efficiency is often hindered by cancer cell repopulation during radiotherapy, which is due to various mechanisms triggered by the loss of tumour cells and involves both stem and differentiated cells. The aim of the current paper is to present *in silico* simulations of radiotherapy schedules on a virtual head and neck tumour grown with biologically realistic kinetic parameters. Using the linear quadratic formalism of cell survival after radiotherapy, altered fractionation schedules employing various treatment breaks for normal tissue recovery are simulated and repopulation mechanism implemented in order to evaluate the impact of various cancer cell contribution on tumour behaviour during irradiation. The model has shown that the timing of treatment breaks is an important factor influencing tumour control in rapidly proliferating tissues such as squamous cell carcinomas of the head and neck. Furthermore, not only stem cells but also differentiated cells, via the mechanism of abortive division, can contribute to malignant cell repopulation during treatment.

***Keywords*—**Radiation, tumour repopulation, squamous cell carcinoma, stem cell.

I. Introduction

REPOPULATION of cancer cells during treatment is one of the leading causes of treatment failure in head and neck carcinomas. The process of repopulation is manifested as a marked increase in tumour growth rate after treatment initiation, and can be up to 15-20 times faster than the growth rate specific to the latency period [1].

There are several mechanisms behind repopulation that have been identified in normal epithelia and are thought to be responsible also for tumour cell repopulation in squamous cell carcinomas of the head and neck [2]. These repopulation mechanisms are linked to both stem cells (such as recruitment of quiescent stem cells, accelerated stem cell division, loss of asymmetrical division of stem cells) and to differentiated cells (such as abortive division and cell recruitment of quiescent differentiated cells) [2]. Cell recruitment refers to the process of cellular re-cycling, whereby quiescent but viable cells re-enter the cell cycle under a stimulus. Through accelerated stem cell division stem cells decrease their cell cycle time while the loss of asymmetrical division leads to symmetrical division, i.e. the creation of two stem cells. Abortive division is a mechanism that refers to the proliferation of differentiated cells rather than stem cells, thus suggests a less powerful effect on overall repopulation. However, in radiotherapy (RT), abortive division can interact with the clinical outcome by counterbalancing the continuous cell loss due to tumour irradiation.

The mechanisms of accelerated stem cell division and abortive division of differentiated cells are controlled by tissue hypoplasia while loss of asymmetrical division is dictated by stem cell depletion. Both stem cells and differentiated cells contribute, to various extents, towards tumour repopulation during treatment.

Treatment of advanced head and neck cancer involves radiotherapy, which is often combined with other treatment modalities such as chemotherapy or immunotherapy. Radiotherapy was given, for several decades in a standard fractionation manner, i.e. a dose of 2 Gy a day, 5 days a week, over 7 weeks. However, the clinical outcome (loco-regional survival, overall survival) with such treatment protocol was poor, mainly due to repopulation of tumour cells during treatment [1]. Therefore, altered fractionation schedules have been suggested in order to overcome malignant repopulation. When managed with a more aggressive schedule, such as accelerated radiotherapy (2 fractions a day, 6 days a week, over 5 weeks), the clinical response of head and neck cancers has improved [3]. The aggressive cancer therapy, however, is also aggressive on the normal tissue, thus treatment breaks are often implemented to allow for the surrounding healthy tissue to recover.

The aim of the present work was to employ an *in silico* head and neck cancer model to illustrate the influence of repopulation, due to both stem and differentiated cells, on treatment break timing during accelerated radiotherapy and to compare the results with the conventionally fractionated radiotherapy.

Given that tumour hypoxia is another important parameter that is responsible for treatment failure, in order to eliminate this bias, the present model considers a normally oxygenated head and neck tumour.

II. Methods

Considering the stochastic nature of tumour growth and its response to radiation, a Monte Carlo computational method has been employed to simulate the growth of a head and neck carcinoma, with biologically realistic parameters [4].

L. G. M. is with the Faculty of Science, University of Oradea, Romania and University of Adelaide, School of Chemistry & Physics, Australia (corresponding author, phone: 40-730-669-502; e-mail: loredana@marcunet.com).

D. M. and S. M. F. are with the Faculty of Science, University of Oradea, Romania.

Cells in the model follow a cell cycle that is conformal to the biological process of mitosis. Thus, cells in mitosis divide into two daughter cells that can be either stem (S), differentiated (P) or non-proliferating (quiescent) cells. These latter cells exit the cell cycle and enter the resting phase, without contributing to tumour proliferation, unless they are triggered back into the cycle as a response to cell loss. The percentage of each cell type was established within the computational model after multiple iterations according to the final biological composition of a malignant tumour. In the model, stem and differentiated cells represent about 12% of the tumour population and have a mean cell cycle time of 33h. Tumour volume doubling time is also in accordance with the literature and has an average value of 52 days.

As a next step, the effect of fractionated radiotherapy has been modeled using the Linear Quadratic formalism of cell survival:

$$S = e^{-(\alpha D + \beta D^2)} \quad (1)$$

where S represents the surviving cells, D is the overall radiation dose and the α and β parameters are tumour specific parameters that characterize radiosensitivity.

Based on the scientific literature, an SF_2 (surviving fraction after a radiation dose of 2 Gy) of 54% has been implemented for head and neck cancer [5].

The aim of the simulation was to follow the behaviour of the virtual tumour on a temporal scale and to analyse tumour response to fractionated radiotherapy when all the mechanisms responsible for repopulation are activated. Based on the RTOG-9030 protocol which has employed an altered fractionation schedule [3] (1.6 Gy/fraction, twice daily, 6 hours apart, 5 days a week and a total number of 42 fractions), three different timings for treatment interruptions have been simulated: after 20, 24 and 28 fractions, respectively. Using the relationship for cell survival (1) and considering the value for the α/β = 8 [6] the surviving fraction for accelerated radiotherapy after 1.6 Gy has been determined and employed by the model. Both stem and differentiated cells have been monitored and their contribution towards tumour growth have been analysed and discussed.

III. Results

The simulation of conventional as well as altered fractionation schedules has led to cell survival curves that illustrate the survival of cancer stem cells and differentiated tumour cells, respectively, following the two different treatment schedules.

Standard (conventional) fractionation of the radiation dose was shown to be inferior to accelerated fractionation, when modeled on head and neck cancers. Fig. 1 shows that due to tumour repopulation during treatment, which is illustrated by the sharp slopes of the surviving curve between consecutive fractions, the standard irradiation protocol cannot eradicate the tumour, while the accelerated radiation schedule is able to control the tumour in a well-oxygenated (normoxic) cell population. It can, therefore, be concluded that non-hypoxic and radioresponsive head and neck tumours can benefit from accelerated radiotherapy. The y-axis of Fig. 1 represents the overall number of stem and differentiated cells, thus the number of tumour cells which could possibly repopulate the primary cancer. Non-proliferating or quiescent cells have not been plotted as they only contribute to the overall tumour mass, without any ability to regrow it. Thus the number of representative cells seems relatively small, due to the small percentage of stem and differentiated cells that constitute a malignant tumour.

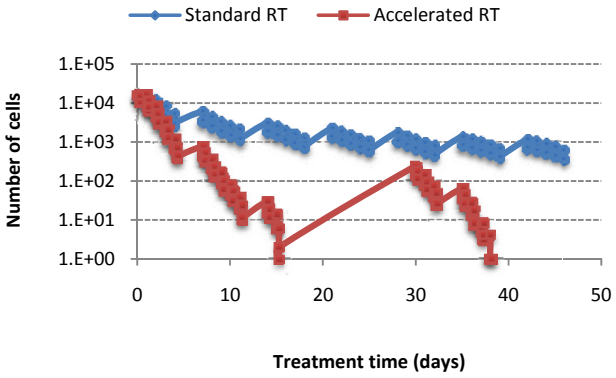

Fig. 1 The effect of standard versus accelerated fractionation radiotherapy on a normoxic head and neck tumour

Due to the activation of repopulation mechanisms during radiotherapy, there is a large increase in the percentage of stem and also differentiated cells that contribute to tumour development. While before radiotherapy the tumour consisted of 5% stem cells (S) and 7% differentiated cells (or finitely proliferating cells, P), during accelerated radiotherapy, both percentages increased drastically, depending also on the timing of treatment breaks. Therefore, the average percent of stem cells varied from 41.3% (break after 20 fractions) to 36.6% (break after 28 fractions), while the average percent of differentiated cells varied from 30.5% (break after 20 fractions) to 33.7% (break after 28 fractions).

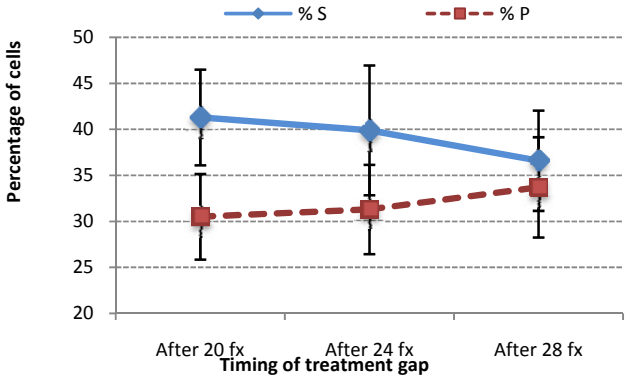

Fig. 2 The influence of treatment gap timing on percentage of cell type [fx = fractions]

An interesting observation is the fact that the percentage of

stem cells decreases with the delay of treatment gap, as shown in Fig. 2. This is because early breaks (after 20 fractions) do not allow sufficient cell kill among the continuously proliferating stem cells to control the tumour (Figs. 3 and 4 – enlarged graph for small number of cells). The behaviour of differentiated cells is just the opposite, to keep a constant cell kill along the treatment.

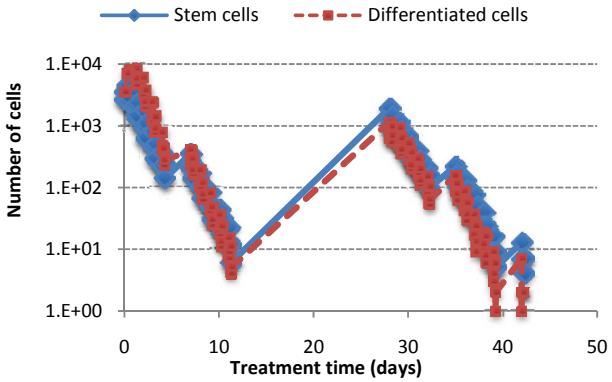

Fig. 3 Surviving curves of stem and differentiated cells under accelerated radiotherapy with treatment gap after 20 fractions

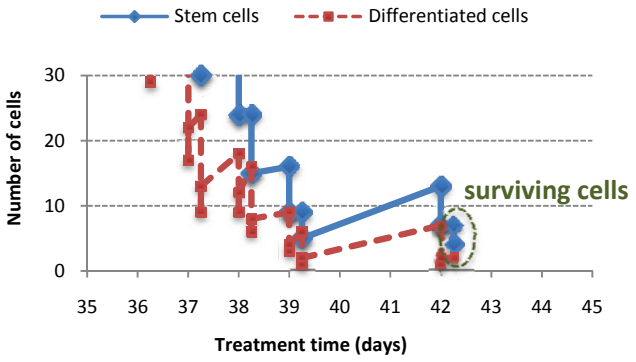

Fig. 4 Enlarged representation of the end part of surviving curve from Fig. 3

When treatment breaks are planned for later, such as after 24 fractions, those extra doses of radiation lead to additional tumour cell kill, which is high enough to hinder repopulation of tumour cells (Figs. 5 and 6). As illustrated in Fig. 5, the number of surviving stem cells after the treatment break is significantly lower (see also Table I) as compared to the schedule that allowed irradiation breaks after 20 fractions. As a consequence, the surviving stem cells could not rebuild the pool of cancer cells during the subsequent doses of radiation. The pattern showed by the surviving differentiated cells is very similar to the one presented by the stem cells. The mechanism of repopulation via abortive division was too weak to counteract the killing effect of radiotherapy on tumour cells.

TABLE I
THE INFLUENCE OF DOSE FRACTIONS ON THE PERCENTAGE OF STEM AND DIFFERENTIATED CELLS

Percentage of cells	Stem (%)	Differentiated (%)
Before radiotherapy	5.27	7.08
After 32 Gy (20 fractions)	3.81	2.26
After 38.4 Gy (24 fractions)	0.31	0.17
After 44.8 Gy (28 fractions)	0.0	0.0

Note: all percentages are calculated out of the total number of initial cells, i.e. from the existing tumour cell pool prior to radiotherapy in order to show the decrease in cell percentage during treatment as compared to the initial tumour composition.

As shown in Table I, the clinical outcome (or the final tumour response) strongly depends on the number of fractions administered before the scheduled treatment break. Therefore, in the present *in silico* model of a virtual, normoxic and radioresponsive head and neck tumour, 50 Gy of radiation given in doses of 1.6 Gy twice daily, can eradicate the tumour, despite repopulation of stem and differentiated cells in between fractions. However, when treatment breaks are scheduled after 32 Gy (i.e. after 20 fractions of 1.6 Gy each), the percentage of stem cells after the break has still a significant value of 3.81% when compared to the initial 5.27%, which is due to repopulation of surviving cells.

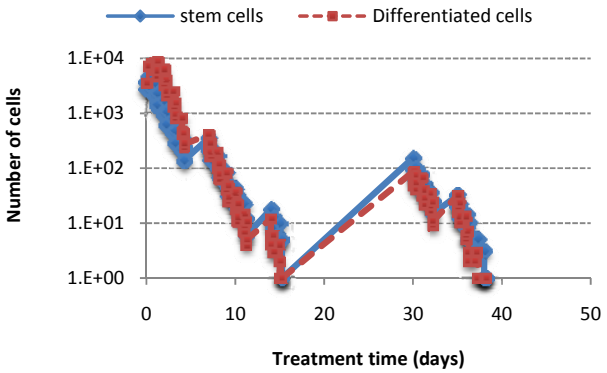

Fig. 5 Surviving curves of stem and differentiated cells under accelerated radiotherapy with treatment gap after 24 fractions

Similar principle applies to differentiated cells. However, given their limited life-time (i.e. limited number of generations); differentiated cells will not be able to regrow the tumour, though repopulation does occur, as shown by all cell survival curves. Stem cells, on the other hand, have a great ability to proliferate indefinitely, fact that leads to a continuous malignant cell repopulation as long as viable stem cells exist within the tumour population.

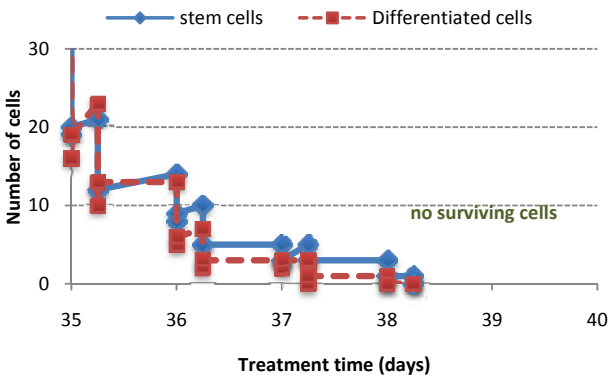

Fig. 6 Enlarged representation of the end part of surviving curve from Fig. 5

IV. Conclusion

Repopulation mechanisms of malignant cells during radiotherapy are important factors that need to be taken into account for a better clinical outcome.

While stem cells are the main contributors towards tumour repopulation during treatment, differentiated cells can also, via abortive division, contribute to malignant cell repopulation in between consecutive doses of radiation. The main difference between these two cell categories is given by the 'immortality' of stem cells which keep proliferating whereas differentiated cells have limited repopulation ability given by the limited number of generations they can survive.

This *in silico* model has shown that the timing of treatment breaks for normal tissue repair and recovery in accelerated radiotherapy is an important factor that influences tumour control in rapidly proliferating tissues such as head and neck carcinomas.

Differentiated cells undergoing abortive division are 'doomed' cells as they eventually cease creating new cells and die out. On the other hand, stem cells are able to regrow the tumour, thus for their eradication there is need for fine adjustments of treatment parameters.

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Dr. Loredana G. Marcu is currently a Professor of Medical Physics at the University of Oradea, Romania and she holds affiliation with the University of Adelaide, Australia. She received her PhD in Medical Physics from the University of Adelaide, and her Masters degree from the Western University of Timisoara, Romania. During her Australian experience she has coordinated the LDR brachytherapy program at the Royal Adelaide Hospital. She was also a TEAP (Training Education and Accreditation Program) preceptor coordinating the medical physics training and education in South Australia.

Her 19 years teaching and research experience at both Australian and Romanian universities has materialised in seven books published on physics, radiobiology and teaching methodologies, respectively, with other two books on Medical Physics being currently in press. She has written 5 textbooks and university lab guides and has over 100 publications and conference presentations worldwide.

Prof. Marcu is a member of ESTRO and RRS. She has been awarded the title "Eminent Scientist of the year 2004" by the International Council for Research and she is the recipient of the "Boyce Worthley award 2006" given by the Australasian College of Physical Scientists and Engineers in Medicine for her achievements in the areas of radiobiology and medical physics. She is the vice-president of the Romanian College of Medical Physicists.