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Biological basis of radiotherapy: where do we stand?

International workshop
Stockholm, 4-5 September 2014

Program and book of abstracts

The Swedish Royal Academy of Sciences,
Lilla Frescativägen 4A
114 18 Stockholm, Sweden
(www.kva.se)

Sponsors

The workshop was generously sponsored by

About the workshop

With a history of more than 100 years, radiation therapy remains one of the main modalities used in the management of cancer together with surgery and chemotherapy.

The progress in treatment planning, image guidance and radiation delivery has led to the appearance of high precision radiotherapy that is a common feature in many clinics. Furthermore, the technological development of functional and molecular techniques for imaging the tumours has opened new possibilities for defining the target and devising the treatment in an innovative manner.

However important questions remain with respect to the relevant clinical and radiobiological aspects.

For radiobiology in particular, progress in research is not accompanied by a quick clinical implementation in spite of its translational character.

Classical radiobiology with its famous 5 R's and the linear-quadratic model for clonogenic survival has been the most influential component of the radiotherapy fractionation schedule design and calculations of isoeffects, while some modern findings do not easily find their way from bench to bedside.

This workshop aims to revisit the old school of radiobiology and identify new findings that have potential to impact on the clinical practice and lead towards the next big leap in clinical radiotherapy: the development of high precision individualised radiotherapy.

Organisers:

Iuliana Toma-Dasu *Medical Radiation Physics, Stockholm University and Karolinska Institutet*
Andrzej Wojcik *Centre for Radiation Protection Research, Stockholm University*
Emely Lindblom *Scientific secretary - Medical Radiation Physics, Stockholm University and Karolinska Institutet*

Venue: The Swedish Royal Academy of Sciences, Lilla Frescativägen 4A
114 18 Stockholm, Sweden (www.kva.se)

Invited speakers:

Jan Bussink - *Radboud University Nijmegen Medical Centre, Nijmegen*

Roger Dale - *Department of Surgery and Cancer, Faculty of Medicine, Imperial College, London*

Alexandru Dasu- *Department of Radiation Physics UHL, Linköping University, Linköping*

Anna Dubrovska - *OncoRay Center for Radiation Research in Oncology, Dresden, Germany*

Marco Durante - *GSI Helmholtzzentrum für Schwerionenforschung, Darmstad*

Eva Forssell-Aronsson - *Sahlgrenska University Hospital, Gothenburg*

Jack Fowler - *University of Wisconsin Medical School, Madison, Wisconsin*

Ester Hammond - *Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology, Oxford*

Jolyon Hendry - *Christie Hospital, Manchester*

Carsten Herskind – *Department of Radiation Oncology, Universitaetsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim*

Michael Joiner – *Department of Radiation Oncology at Wayne State University School of Medicine in Detroit, Michigan*

Wolfgang Sauerwein – *University Clinics Essen, Essen*

Albert Siegbahn – *Medical Radiation Physics, Stockholm University and Karolinska Institute*

Klaus Trott - *University of Pavia, Italy*

Conchita Vens - *Experimental Therapy Division at the Netherlands Cancer Institute, Amsterdam*

PROGRAM

September 4, 2014

Introductory lecture

08:30-09:00	Radiobiology of clinical fractionated radiotherapy – Textbook versus new knowledge	Iuliana Toma-Dasu

Session 1 - What is the target of radiotherapy?

Chairperson: Rolf Lewensohn

09:00-09:40	The importance of tumour stem cells for radiotherapy	Anna Dubrovska
09:40-10:20	The importance of normal tissue stem cells for radiotherapy	Klaus Trott
Coffee break		
10:40-11:20	The importance of tumour environment for radiotherapy	Jan Bussink
11:20-12:00	The development of beam-grid based radiosurgery	Albert Siegbahn
Lunch break		

Session 2 - The classic 4 Rs of radiotherapy – are they still valid?

Chairperson: Mats Harms-Ringdahl

13:00-13:40	Repair of sublethal damage in tumour and normal tissue cells	Conchita Vens
13:40-14:20	Tumour cells reassortment within the cell cycle (including check points and cell cycle arrest)	Carsten Herskind
Coffee break		
14:40-15:20	Proliferation and accelerated repopulation in tumour and normal tissue	Jolyon Hendry
15:20-16:00	Hypoxia, reoxygenation and radiation sensitivity	Ester Hammond
Coffee break		
16:30-17:30	**General discussion**	Moderator: Claes Mercke

19:00 Dinner at Karolinska Hospital, CCK building, floor 5 (see maps). Busses will be provided to transport participants to Karolinska.

September 5, 2014

Session 3 - The LQ model and its parameters

Chairperson: Per Nilsson

09:00-09:40	Radiobiological basis of the LQ model	Mike Joiner
09:40-10:20	The radiobiological modelling challenges of 21st century radiotherapy	Roger Dale
Coffee break		
10:40-11:20	LQ parameters – Does one size fit all? Heterogeneity in parameters versus one single set of parameters for all the cells in the tumour and normal tissue	Alexandru Dasu
11:20-12:00	Is there an optimal treatment time and fractionated schedule?	Jack Fowler
Lunch break		

Session 4 – New/old treatment modalities

Chairperson: Bo Stenerlöw

13:00-13:40	Biological basis of targeted radiotherapy	Eva Forssell-Aronsson
13:40-14:20	Biological basis of brachytherapy	Andrzej Wojcik
Coffee break		
14:40-15:20	Biological basis of hadron therapy	Marco Durante
15:20-16:00	Biological basis of neutron therapy	Wolfgang Sauerwein
Coffee break		
16:30-17:30	**General discussion and concluding remarks**	Moderator: Mike Joiner

17:30 – end of meeting

The importance of the tumor microenvironment for radiotherapy

Jan Bussink

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The microenvironment consists of a complex symbiosis of various closely interacting cells. For growth, the tumor is depending on nutrients and oxygen from the 'host' system. Also, waste products are discarded towards that same 'host'. Sensitivity and resistance to radiotherapy is independently connected to the microenvironment. The complex and often chaotic vascular architecture, induced by tumor cells itself, leads to heterogeneous distribution of hypoxic cells, proliferating cells immune cells etc. Consequently insufficient oxygenation of tumor cells makes them more resistant to ionizing radiation. Treatment by irradiation not only depends on the microenvironment, it also severely affects the microenvironment. Radiotherapy leads to changes in the balance with respect to vascular damage and vascular remodeling, hypoxia and reoxygenation, immune suppression and immune activation. The position of this balance contributes to the faith of a tumor.

The radiobiological modelling challenges of 21st century radiotherapy

Roger Dale

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Radiotherapy remains a successful and cost-effective treatment modality for a wide range of cancers and, despite occasional suggestions of its imminent demise, new and exciting techniques continue to be developed. Many of the newer treatments represent considerable departures from the types of radiotherapy which have been practiced for over 100 years, meaning that the bedrock of previous clinical experience may not always be relevant in guiding the optimal application of the new methods. The requirement for a sound radiobiology base from which to quantitatively guide the optimal application of the emergent technologies is thus stronger than ever before.

This talk will review some of the challenges posed by new radiotherapy practices and will highlight those areas where current radiobiological understanding is inadequate or in need of further refinement. Even in cases where current radiobiological models are known to be inadequate descriptors of events at the macroscopic (i.e. clinically observable) level, it is important not to lose sight of the fact that, at the microscopic level, the bio-physical origins of the models may be sound. This suggests that translational research has a major role to play in adapting radiobiology to the requirements of 21st Century treatment practice and that there is an urgent need to promote and support such research and disseminate and implement findings.

To cultivate an appreciation of why such research is required there is a parallel requirement for the next generation of radiation oncologists and radiation scientists to understand both the limitations and potential of radiobiology and, for this reason, on-going education programmes in radiobiology will be of key importance.

LQ parameters for modelling - Does one size fit all?

Alexandru Dasu

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LQ modelling with parameters derived from populations of patients has been used for many years for accurate isoeffect calculations regarding the response of tumours or the tolerance of late and acute reacting normal tissues from many clinically-used fractionated schedules. The parameters used are thought to describe the “average patient”, but in reality rather broad interval of values may be found in individuals in the investigated populations and therefore the value of pre-treatment derived parameters to predict treatment response has often been debated. Furthermore, clinical outcome can often be modelled by assuming quite extreme single values for the parameters, while experimentally-derived parameters require assumptions about inter-patient heterogeneity as heterogeneity in radiation sensitivity or tumour cell population influences the position and the slope of the tumour control probability curve and therefore directly impacts upon predictions for treatment outcome. This lecture aims to explore the question of parameters to be used for LQ modelling, the opportunity of using parameters relevant to individuals or the average, as well as the impact of accounting for inter-patient heterogeneity in tumour control probability predictions or the search for an optimum overall treatment time.

Radiotherapy and cancer stem cells

Anna Dubrovska

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Radiotherapy is a curative treatment option in many types of tumors. Nevertheless, cancer patients can relapse after radiation therapy. The radiotherapy failure might be related to cancer stem cell (CSC) population which was not completely sterilized during the treatment and can provoke tumor re-growth [1-3]. The proportion of CSCs has a high intertumoral variability. Current findings suggest that estimation of the number of CSCs in pre-therapeutic tumor biopsies might be predictive of tumor radiocurability in some types of cancer including cervical squamous cell carcinoma (CSCC), head and neck squamous cell carcinoma (HNSCC), rectal carcinoma and glioma patients [2, 3]. Despite some limitations of these studies, they have paved an avenue for future implication of CSC-related predictive biomarkers for radiotherapy. In addition to the impact of CSC density on tumor radiocurability, recent experimental reports suggest a number of different intrinsic and extrinsic adaptations that confer tumor radioresistance and which also occur in CSC populations, including quiescence, increased DNA repair capability, activation of the cell survival pathways and scavenging of the reactive oxygen species (ROS) that can induce DNA damage [3-5]. A broad variety of the microenvironmental niches as well as genetic and epigenetic changes of tumor cells during tumor development make cellular radiosensitivity dynamic in nature [3, 5-7]. The talk will review the role of CSCs in tumor radiocurability and their implication in the development of predictive assay for radiotherapy. The limitations of the current models to study CSC properties including their putative radioresistance will be also discussed.

References

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Biological basis of hadron therapy

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The use of high-energy charged particles in radiotherapy was proposed several years ago, both for their physical and radiobiological advantages compared to X-rays. Particle therapy is now generally considered a cutting-edge technology with a tremendous potential for several cancers associated to poor prognosis, such as pancreas, locally recurrent rectal cancer, and lung. Protons and carbon ions have been used for treating many different solid cancers, and several new centers with large accelerators are under construction. Meanwhile, tremendous technological improvements in image-guided radiation therapy (IGRT) paved the way to hypofractionation (down to high-dose, single fraction treatments) of X-rays for both cranial (stereotactic radiosurgery) and extracranial (stereotactive ablative radiotherapy) targets. These new technologies can be competitive with surgery, for example in lung cancer. Can particle therapy produce better results in radiosurgery and eventually become an alternative to surgery? The talk will try to address this question and present research challenges in medical physics and radiobiology.

Optimum Overall Times for Advanced Head and Neck Radiotherapy

Jack F. Fowler

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Radiotherapy has a history of more than 100 years of the impressive record of being second only to surgery in the treatment of cancers. It is a non-invasive technique that relies on improving the localisation of the target, the dose delivery to smaller and smaller volumes and on detecting differences in biological properties, including genetic properties, to increase the outcome. Nevertheless, a central issue of modern radiotherapy remains finding the best optimal schedules that can be obtained from radiation itself. Two different constraints govern any radiation oncology treatment, one that tries to make the overall time longer and the other that tries to make it shorter. The natural constraint that tends to lengthen the overall treatment time is the successive addition of a small dose per day of treatment, which might be 2 Gy per fraction per treatment day for example. The constraint for limiting the length of the overall time comes from the repopulation rate of the malignant cells of the tumour during the treatment. It is from balancing these two opposing forces acting on the treatment that leads to an optimum overall time for the radiotherapy of rapidly proliferating tumours like those of the head and neck, keeping also in mind the constraints set by the normal tissues. Thus, the dose to the late normal tissues must not exceed the total tolerance dose equivalent to 70 Gy in 35 fractions, while acute mucosal reactions have a total tolerance dose equivalent to 51.3 Gy in 2 Gy fractions. Several strategies have been proposed to fulfil these constraints including delivering one or several fractions per day. This lecture focuses on obtaining and exploring the best optimal schedules in modern radiation therapy of head and neck cancers. Although it has become clear human papilloma virus cancers do better when treated with radiotherapy than those not infected with the virus that does not alter the necessity of using the best radiotherapy schedule that can be obtained with respect to its optimum overall time. This makes the search for the optimum overall times an actual problem of modern radiotherapy.

Targeting hypoxic tumour cells through the DNA damage response

Ester M. Hammond

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Tumour hypoxia is a common feature of solid tumours and negatively impacts patient prognosis. The increased radioresistance of hypoxic cells significantly decreases the efficacy of radiotherapy. The most severely hypoxic regions of a tumour are also the most radioresistant (radiobiologic hypoxia). In response to radiobiologic hypoxia a DNA damage response (DDR), including ATM and ATR, is induced. Our studies have demonstrated that inhibition of key factors in the DDR, for example ATR, ATM and Chk1, can sensitise cells to hypoxia and subsequent reoxygenation. Most importantly, we have shown that a number of DDR inhibitors increase the radiosensitivity of hypoxic cells. These combinations offer new strategies to target tumour hypoxia. Most recently, we have developed a series of hypoxia-activated prodrugs. These agents are inactive at normal oxygen levels but in conditions of low oxygen are reduced to release an active inhibitor. The targeting of Chk1 inhibition to hypoxic cells will be discussed.

Repopulation concepts in irradiated normal tissues and tumours

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Stem cells are the best cells you will ever have to repopulate depleted normal tissues. Lineages of stem cells, daughter differentiating cells (transit cells) and mature functional cells have been characterised in many tissues. Some lineages are short (breast, skin), and others long (bone marrow, spermatogenesis). The Labelling Index in these tissues is governed mainly by the predominant dividing transit cells, not by the stem cells. Up to 6 surface biomarkers have been found necessary to identify stem cells in various tissues. The stem-cell self-renewal probability (p) is 0.5 in steady-state (no net growth), and about 0.6 in exponential growth after much radiation-induced depletion. During 2 Gy daily fractionation, there is a latency interval followed by repopulation equivalent to about 1 Gy/day (D_{prolif}) in human oral mucosa.

In untreated human tumours, a short volume doubling time of 40 days could be explained by a cell cycle time of 2 days, a growth fraction of 50% (giving a potential doubling time of 4 days), and a cell loss factor of 90%, if all tumour cells were malignant. During 2 Gy daily fractionation, D_{prolif} is 0.6-0.8 Gy/day for head & neck tumours. This has led to guidelines for avoiding detrimental interruptions in treatment, and to new trials of accelerated schedules. There has been renewed interest in the concept of cancer stem cells (CSC), where tumours retain some of the lineage characteristics of their tissue of origin. This comes from the histological grading of differentiation status, and from many experimental studies of target cell number and other characteristics. The concept suggests that well-differentiated tumours may show some of the accelerated repopulation characteristic of normal tissues and should benefit more from accelerated schedules, whereas longer conventional schedules could be used for poorly-differentiated tumours. Also, it opens up new developments based on target cell imaging linked to dose distributions, and combined treatments to overcome CSC resistance. Identification of more CSC biomarkers would help these approaches.

Tumor cell reassortment within the cell cycle (including checkpoints and cell cycle arrest)

Carsten Herskind

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The survival advantage of cells irradiated in radioresistant cell-cycle phases makes reassortment of the surviving cells over the more radiosensitive cell-cycle phases part of the rationale for fractionated radiotherapy. While it has not been possible to exploit the partial synchronization of tumour cells in clinical practice, understanding the regulation of cell-cycle progression after irradiation may nevertheless be important for changes in fraction size and may potentially be used to manipulate the radiosensitivity of cancer cells. The DNA damage response involves cell-cycle arrest at different checkpoints, repair of damage, as well as the decision to resume proliferation or prevent cell reproduction if residual damage cannot be repaired or is misrepaired. Thus checkpoints are present before and during replication in the G1/S and S phases, and before mitosis in the G2/M phase. However, novel sophisticated techniques in cell-cycle labeling and signal transduction have revealed several molecularly distinct checkpoints differing in kinetics, efficiency, and dose dependence, which can be suppressed by small molecule inhibitors or by RNA interference. Thus, the most conserved checkpoint in G2/M has been shown to be regulated by at least three different pathways. The current understanding of the DNA damage checkpoints in S, G2/M, and G1/S, will be reviewed with special emphasis on their sensitivity, imperfections and the release of cells from the various checkpoints. Furthermore the potential for modulating radiosensitivity will be discussed.

Radiobiological basis of the LQ model – Is there one…?

Michael C. Joiner
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Biological effect and physical dose are non-linearly related. This was implicitly recognized very early in the development of radiotherapy, and led to the power-law equations which dominated thinking for several decades. Deficiencies in the power-law description were identified even as early as the 1960's, and in the 1970's and 80's a massive amount of experimental work on many newly-developed models of normal-tissue injury in rodents and pigs, showed that –ln(*Surviving Fraction*) is much better described by a second-order polynomial in dose, the well-known Linear-Quadratic (LQ) equation. The LQ description has since been thoroughly tested in the clinical domain. LQ is the simplest mathematical description of a non-linear relationship and though empirical in nature, it has nevertheless been subject to many attempts to connect with our understanding of how radiation injury is produced and repaired at the cell and molecular level. Yet any meaningful and clinically useful link in this respect has remained largely elusive.

LQ deals specifically with the relationship between total dose and dose per fraction, and with interfraction interval using the Incomplete-Repair derivative model. The relationship between total dose and overall treatment time, originally included in the power-law models in a manner that didn't match actual clinical observations, is an even more complex relationship dependent on the different underlying radiobiology of different tissues even within the apparently same category of early-reacting or late-reacting tissues, distinguished by respectively a "high" or "low" ratio of α/β in the LQ equation. Overall time is therefore better handled independently of LQ. Prediction of tolerance to retreatment is an even more complex issue where our radiobiological understanding is earlier in development and useful models are not yet to hand.

A straightforward but untested hypothesis for the different α/β values for early- and late-reacting tissues, is that a naturally low α/β for a target cell population is smoothed out to a higher value as the sum of the responses of different proliferative subpopulations, and different phases of the cell cycle that these are in. This same explanation could also be applied

to the responses of malignancies in the lung and head and neck, also adding in the additional response variation of cells at various levels of hypoxia in these sites. Of note is the recent connection made between outcome of radiotherapy and HPV status in oropharyngeal cancers, which implies a possible difference in treatment strategy between these tumor subtypes and could also explain the high α/β of head and neck cancer overall as the sum of the responses of the different cancer subtypes (HPV + and −) which could both have low α/β but different radiosensitivity. Importantly, it is now evident that in some malignancies, notably prostate and breast, clinical data do indeed indicate a low α/β which might also reflect more uniformity in response perhaps more characteristic of lower proliferative or early-stage disease. This has resulted in new efforts to test hypofractionation which have also been enabled by the better dose localization achievable with image-guided Volumetric Modulated Arc Therapy.

There is evidence that the LQ model becomes less reliable at doses per fraction < 1 Gy, due to possible low-dose hyper-radiosensitivity, and also at > 6 Gy per fraction for reasons not yet understood. Though, it is axiomatic that LQ must indeed overestimate effect at very high doses per fraction because the effective D0 would become unrealistically low. This makes the outcome of hypofractionated regimes less predictable: using LQ at high doses per fraction would be playing safe in predicting toxicity of hypofractionation, while overestimating the effect on the target malignancy, noting that possible hypoxia in a tumor could also limit the effectiveness of large dose fractions.

The development of a beam-grid based radiosurgery

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At the onset of the lecture, the historical development of radiation grid therapy will be presented. Thereafter, the dose volume effect and the potential benefit of using beam arrays with small beam elements will be discussed. The in vivo microbeam experiments with rodents, undertaken for the first time in the 1950s as a part of the United States space exploration program, showed that there is nearly no observable functional damage of central nervous system tissue after such irradiations. In an attempt to explore this large tissue tolerance to microscopic beams, x-ray microbeam-grid radiation therapy preclinical trials began in the synchrotron laboratory at Brookhaven National Laboratory in the late 1990s. The idea was to irradiate a cancer target from different directions up to high doses with spatially-fractionated x-ray beams. At this point in time, nearly parallel x-ray beams of sufficiently high energy (for depth penetration) and fluence (to avoid motion issues) had become available which was deemed necessary for this kind of treatment. Narrow, micrometer-wide x-ray beams with a close separation form the beam array used for this therapy. In recent years, this method has been developed and tested at different synchrotron laboratories on four different continents. To date, MRT has been evaluated mainly with small experimental animals, e.g. rodents, some of them bearing a transplanted tumour. Data from these tests will be presented in this lecture. MRT preclinical trials ultimately aiming toward treating humans have recently moved forward with treatments of larger animals, e.g. cats, with spontaneous tumours. Some tests have also been performed by different groups with beam arrays containing other types of particles, such as with grids of proton or carbon-ion beams. With ions the additional possibility exists to spare sensitive structures posterior to the target in the beam direction.

The importance of normal tissue stem cells for radiotherapy

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The existence and the radiosensitivity of normal tissue stem cells and their role in the pathogenesis of normal tissue damage was first demonstrated in the bone marrow. Fatal bone marrow hypoplasia and subsequent agranulocytosis is related to sterilization of about 99.9% of bone marrow stem cells. The clinical consequences can be avoided by intravenous injection of a sufficient number of viable bone marrow stem cells into the irradiated animal, which not only gives additional proof of the role of bone marrow stem cell sterilization in the pathogenesis of bone marrow failure but also was the basis of clinical application of stem cell transplantation in the treatment of radiation-induced hypoplasia. Extrapolating these radiobiological data to all other normal organs and tissues, inactivation of normal tissue stem cells (called tissue rescuing units) was widely taken as the main pathogenic pathway leading to normal tissue damage from radiotherapy. However, experimental and clinical radiobiology research demonstrated that whereas in early normal tissue damage of some tissues such as oral mucositis and intestinal mucositis, radiation damage of stem cells play some role, the pathogenesis of late normal tissue damage is much more complex and stem cells appear to play no significant role. Each type of late complication after radiotherapy depends on its own specific mechanism which is triggered by the radiation exposure of particular structures or subvolumes of the respective organ at risk. The radiobiological mechanisms which are involved in the resulting pathogenesis differ between the different complications, even in the same organ which follow different dose effect relationships and show different dependence on dose per fraction, overall treatment time and anatomical dose distribution within the subvolume at risk. The different mechanisms can be classified as:

1. single cell effects such as “cell death”, sterilisation of stem cells, inhibition of proliferation,
2. tissue effects which depend on the interaction between different cells and cell populations within organs or between organs such as inflammation or differentiation,
3. effects which result from alterations of tissue structure and function such as vascular injury,

4. other, less well defined functional changes such as alterations in neuromuscular function or immunological responses.

The most important role normal tissue stem cells are likely to play in the pathogenesis of late complications of radiotherapy is related to the induction of second cancers, however even there their role is controversially discussed.

This work was performed as part of the FP7 supported research project ALLEGRO.

Biological basis of brachytherapy

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Brachytherapy is the delivery of radiation therapy using sealed sources that are placed as close as possible to the site to be treated. The major advantages of brachytherapy lie in the physics of dose distribution around the source, which results in a high concentration of dose around the source. A disadvantage lies in the need to access the tumour with an operative procedure requiring very skilled personnel to undertake the treatment.

Brachytherapy is most often used as boost to external beam radiotherapy in order to increase the dose to the tumour without increasing the dose to normal tissues. This approach is understandable and it is often claimed that the radiobiology of brachytherapy is governed by exactly the same principles as external beam therapy (the 4 Rs of radiotherapy).

In the treatment of prostate cancer brachytherapy is increasingly used as monotherapy. This became possible thanks to the development of trans-rectal ultrasound (TRUS) to precisely position the radiation sources. Initially, the monotherapy was used to treat low and intermediate risk group patients with I-125 seeds yielding a low dose rate (LDR). At the same time it became increasingly evident that the α/β value for prostate cancer is very low, indicating the possibility of hypofractionation. Warning statements were published not to go below a few fractions in order to maintain the benefit of reoxygenation. Yoshioka and colleagues were the first to test high dose rate (HDR) brachytherapy as monotherapy for prostate cancer. The good results were confirmed by others and recent years saw several successful attempts to treat prostate cancer by a single HDR fraction.

The implications of this development for the understanding of the biological basis of radiotherapy will be discussed.

Three New Mechanistic Radiobiological Models for Cell Response to Radiation

Dževad Belkić & Karen Belkić: Department of Oncology-Pathology, Karolinska Institutet

Second-order kinetics are utilized to describe the three main mechanisms for surviving fractions of cells after irradiation. These are a direct yield of lethal lesions by single event inactivation, metabolic repair of radiation lesions and transformation of sublethal to lethal lesions by further irradiations. The mass action law is employed to set up a system of time-dependent, non-linear differential equations for average molar concentrations of the invoked species, such as DNA substrates as lesions, enzyme repair molecules, a pool of intracellular repair substances, etc. The analytical solutions of these coupled rate equations are derived. Three new radiobiological models, based on the concept of a pool of repair molecules and Michaelis-Menten enzyme catalysis, each having three dose-range biologically interpretable parameters of clinical relevance, are analyzed from two different systems of rate equations that are solved by extracting the concentration of lethal lesions whose time development is governed by the said three mechanisms. The Poisson distribution of the derived concentrations of lethal lesions gives the closed expression for the cell surviving fractions after irradiation. Exploiting the derived asymptotes of the analytical solutions, the three new dose-effect curves are shown to exhibit shoulders at intermediate doses preceded by the exponential cell kill with a non-zero initial slope and followed by the exponential fall-off with the reciprocal of the mean lethal dose as the final slope. All three dose regions are universally as well as smoothly interconnected in the ensuing surviving fractions. The proposed three radiobiological models are equally valid for low- and high-dose per fraction treatment regimens. It is expected that these new models will find the most needed applications in hypofractionation treatment schedules (stereotactic radiosurgery, stereotactic body radiotherapy) for which the low-dose linear-quadratic model is known to be seriously challenged.

Improved Distinction by MR Spectroscopy of Suspicious Lesions after Radiation Therapy among Children with Primary Brain Tumors

Karen Belkić and Dževad Belkić
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Brain tumors are the leading cause of cancer-related deaths among children. Radiation therapy (RT) is indicated for unresectable and/or malignant brain tumors. After RT, primary brain tumors frequently recur. Changes in brain tissue can also be provoked by RT, and these are difficult to distinguish from recurrent tumor using magnetic resonance imaging (MRI). Increasing attention in pediatric neuro-oncology has been given to magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI). We systematically examine MRS and MRSI data concerning distinction of recurrent brain tumor versus radiation necrosis post-RT among children with primary brain tumors. From the limited available data, it appears that, among children, choline-to-creatine ratios > 2 indicate tumor recurrence whereas choline-to-creatine ratios < 2 are associated with radiation necrosis. More extensive analysis of the adult plus pediatric population, however, has indicated that, while helpful, metabolite ratios do not provide unequivocal distinction between recurrent tumor and radiation necrosis. These limitations are likely related to reliance upon sub-optimal signal processing methods conventionally used within MRS and MRSI. We demonstrate that conventional Fourier processing using clinical MR scanners may generate inaccurate metabolite ratios. Optimized signal processing via the fast Padé transform not only yields accurate metabolite ratios with very high resolution, but also provides reliable quantification of over 20 brain metabolites. Padé-optimized MRS and MRSI could thereby facilitate differential diagnosis of new lesions appearing on MRI among children treated for primary brain tumors. These advantages of the fast Padé transform are more broadly applicable to MRS and MRSI within pediatric neuro-oncology and beyond.

Gender-related differences in pathological and clinical tumour response based on immunohistochemical proteins expression in rectal cancer patients treated with short course of preoperative radiotherapy

Anna Gasinska[1], Agnieszka Adamczyk[1], Joanna Niemiec[1], Beata Biesaga[1], Zbigniew Darasz[2], Jan Skolyszewski[3]

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Introduction Assessment of prognostic value of pretreatment expression of six proteins in rectal cancer for early pathological tumour response (pTR), clinical tumour response (CTR) to preoperative radiotherapy (RT), and the potential difference between these parameters depending on patient gender.

Material and Methods 111 patients were treated with short preoperative course of RT (SCRT) with 5 Gy dose per fraction during 5 days, followed by surgery 3 to 53 days (mean 21 days) later. Expression of GLUT-1, Ku70, BCL-2, P53 proteins was assessed immunohistochemically. Tumour regression after RT was assessed by surgeons at the time of operation (CTR), and by pathologist on the excised tumour mass (pTR).

Results There were 76 men and 35 women. There were 27 cTNM stage I, 69 stage II and 15 stage III tumours. We found 26 well-differentiated, 80 moderately-differentiated and 3 poorly-differentiated tumours. Significant differences in Ki-67, GLUT-1, Ku 70 and BCL-2 expression between male and female tumours were observed for pathological stage (pTNM) and grade. Association between proteins expression and pTNM, pTR and CTR was analysed separately for short (≤ 15 days) and long (> 15 days) break between RT and surgery and males and female patients. For SCRT with short break none protein was significantly related to pTNM, and for pTR higher Ki-67 and lower BCL-2 expression were correlated with pTR. In the male subgroup, BCL-2 overexpression was predictive. For SCRT with long break none of the proteins was predictive for pTR but Ki-67, Ku70 (in female subgroup) and BCL-2 expression were positively correlated with pTNM, what was shown for the first time. BCL-2 overexpression was associated with CTR in female subgroup, only.

Conclusion In SCRT long break in the treatment should be avoided because correlation between Ki-67, KU70 and BCL-2 expression and pTNM after RT might indicate tumour progression reflecting tumour cell repopulation.

An association between low-dose hyper-radiosensitivity and the early G2-phase checkpoint

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The phenomenon of low-dose hyper-radiosensitivity (HRS) is an effect in which cells die from excessive sensitivity to low doses (<0.3 Gy) of ionizing radiation but become more resistant (induced radioresistance, IRR) to larger doses. The mechanisms regulating HRS/IRR biology seem to be complicated and are continuously investigated. According to the proposed hypothesis, HRS reflects the apoptotic death of cells as a consequence of ineffective early G2-phase arrest of cells irradiated in G2 phase. According to some data the checkpoint is activated after exposure to a threshold dose of 0.2 Gy and above.
In our previous study, using flow cytometry-based clonogenic survival assay, the HRS response was demonstrated for normal fibroblasts (asynchronous and G2-phase enriched cell populations) of 4 of the 25 cancer patients investigated (IJRBP 2014, 88, 369-376). This gave us a reason to examine the mechanism underlying HRS phenomenon. The aim of our study was to determine an association between low-dose hyper-radiosensitivity and the early G2-phase checkpoint activation. The response of the checkpoint was examined by assessment of the progression of irradiated cells into mitosis using the mitotic marker, phosphorylated histone H3. The number of mitotic cells as a function of radiation dose was assessed for the asynchronous fibroblasts of 4 HRS-positive and 4 HRS-negative patients.
After irradiation with doses lower than 0.3 Gy HRS-positive fibroblasts continued to enter mitosis and, as a result, no reduction in mitotic ratio was observed. The dose over which the number of mitotic cells started to decrease corresponded well with the transition dose (specific for each HRS-positive patient) required to overcome HRS. In contrast, HRS-negative fibroblasts exhibited an immediate post-irradiation decrease in the mitotic ratio even at the lowest doses.

The data provides support to the existing hypothesis suggesting the relationship between the HRS response and failure of the early G2-phase checkpoint.

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Aberrant DNA damage response and receptor tyrosine kinase signaling in non-small cell lung cancer tumor initiating cells

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Increasing evidence suggest that tumor initiating cells (TICs), also called cancer stem cells (CSC), are partly responsible for resistance to DNA-damaging treatment. Using a model of sphere-forming non-small cell lung cancer (NSCLC) cells to characterize the specific properties of TICs, we show that they displayed reduced apoptotic response and less pronounced cell cycle arrest after DNA-damaging treatment e.g. irradiation (IR) and cisplatin. TICs also showed altered DNA repair signaling with diminished DNA damage-induced phosphorylation of ATM and KAP1. Receptor tyrosine kinases (RTK) are important regulators of intracellular signal transduction pathways which influence tumor cell sensitivity to DNA damaging treatments. Here we examined if NSCLC TICs show an altered basal and IR-induced activation of 28 RTKs and 11 important signaling nodes as compared to bulk tumor cells using PathScan RTK Signaling Antibody Array Kit. TICs displayed decreased basal phosphorylation of insulin-like growth factor 1 receptor (IGF-1R) and signal transducer and activator of transcription 1 (STAT1) (Tyr701) as compared to bulk cells. Total IGF-1R levels were significantly lower in NSCLC TIC as compared to bulk cells. A higher degree of extracellular signal-regulated kinase (ERK) phosphorylation was evident in TIC and concordantly MEK inhibition reduced TIC viability. Moreover, MEK inhibition also decreased clonogenicity upon IR suggesting that MEK and downstream signaling impart on TIC radiation response. In conclusion, we demonstrate that NSCLC TICs have both altered DNA damage signaling response as well as altered RTK activation which may influence their resistance to DNA damaging treatments such as IR.

The influence of repopulation mechanisms on treatment gap timing in head and neck cancer radiotherapy

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Background: In head and neck cancers tumour repopulation and hypoxia are the leading cause of treatment failure. Various mechanisms behind repopulation have been identified: recruitment of quiescent cells, accelerated stem cell division, loss of asymmetrical division of stem cells and abortive division. The latter mechanism refers to the proliferation of differentiated cells rather than stem cells, however, it can still contribute towards overall repopulation. Advanced head and neck cancers are often managed with altered fractionation schedules, such as accelerated radiotherapy, in order to overcome malignant repopulation. Treatment breaks can be employed to allow normal tissue recovery. The aim of the present work is to illustrate the influence of repopulation due to both stem and differentiated cells on treatment break timing during accelerated radiotherapy in head and neck carcinomas.

Methods: A Monte Carlo computational method has been employed to simulate the growth of a head and neck carcinoma, with biologically realistic parameters. In the model, stem and differentiated cells represent about 16% of the tumour population and have a mean cell cycle time of 33h. The aim of the simulation was to follow the behaviour of the virtual tumour on a temporal scale and to analyse tumour response to altered fractionation radiotherapy when all the mechanisms responsible for repopulation are activated. Based on a RTOG schedule (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 using the Linear Quadratic model: after 20, 24 and 28 fractions, respectively. Both stem and differentiated cell have been monitored and their contribution towards tumour growth analysed and discussed.

Results: 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 6% stem cells and 10% differentiated cells, 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). An interesting observation is the fact that the percentage of stem cells decreases with the delay of treatment gap. This is because early breaks (after 20 fractions) do not allow sufficient cell kill among the continuously proliferating stem cells to control the tumour. The behaviour of differentiated cells is just the opposite, to keep a constant cell kill along the treatment.

Conclusions: The model has shown that the timing of treatment breaks is an important factor influencing tumour control in rapidly proliferating tissues. Not only stem cells but also differentiated cells, via abortive division, can contribute to malignant cell repopulation during treatment. 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.

Cluster pattern analysis of energy deposition sites for protons, other light ions and brachytherapy sources

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The spatial pattern of energy deposition (ED) sites formed by the interactions of ionizing particles and their secondary electrons within a cell nucleus could determine the severity of the initial damage to the DNA and hence influence the relative biological effect (RBE) of the actual radiation type. RBE data is needed to improve the accuracy of treatment planning due to the many concurrent radiation qualities usually characterized by their linear energy transfer (LET) which is an imprecise predictor of RBE. It could be advantageous if RBE could be predicted in a reliable way. The aim of this work was to analyse the cluster patterns of EDs formed by proton and other light ions as well as five of the most frequently used brachytherapy sources, 103Pd, 125I, 192Ir, 137Cs and 60Co. The ED data was obtained by performing event-by-event Monte Carlo simulations with the LIonTrack and PENELOPE radiation transport codes. A novel cluster method was developed which does not bias radiation clustering properties towards preselected cluster scoring volumes. The number of clusters with cluster order (number of EDs contained in the cluster) larger than two increases with decreasing atomic number for ions with the same LET (25.7 eV nm-1). The ratio of cluster order distributions for the brachytherapy sources reveals that the two lower mean photon energy sources (103Pd, and 125I) differ by as much as 15% with respect to 60Co, while the ratio differs by only 2% for the two sources with higher mean photon energy (192Ir, and 137Cs). Our results encourage the use of cluster order distributions as a foundation for RBE analysis since it can discern differences between ion species not predicted by LET variations.

A study of V79 cell survival after for proton and carbon ion beams as represented by the parameters of Katz' track structure model.

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Katz's theory of cellular track structure (1) is an amorphous analytical model which applies a set of four cellular parameters representing survival of a given cell line after ion irradiation. Usually the values of these parameters are best fitted to a full set of experimentally measured survival curves available for a variety of ions. Once fitted, using these parameter values and the analytical formulae of the model calculations, cellular survival curves and RBE may be predicted for that cell line after irradiation by any ion, including mixed ion fields. While it is known that the Katz model parameters fitted to heavier ion data may yield unsatisfactory predictions of proton response, to our knowledge, no comprehensive data set which includes proton and heavier ion irradiations, measured in one laboratory, has been published. To study the consistency of evaluating parameters of this model from different sets of data obtained for the same cell line and different ions, measured at different laboratories, we have fitted model parameters to a set of carbon-irradiated V79 cells, published by Furusawa et al. (2), and to a set of proton-irradiated V79 cells, published by Wouters et al. (3), separately. We found that values of model parameters best fitted to the carbon data of Furusawa et al. yielded predictions of V79 survival after proton irradiation which did not match the V79 proton data of Wouters et al. Fitting parameters to both sets combined did not improve the accuracy of model predictions of the proton response. This suggests that for increased accuracy of a therapy planning system based on Katz's model, different sets of parameters may need to be used to represent cell survival after proton irradiation from those representing survival of this cell line after heavier ions, up to and including carbon irradiation.

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Genetic variants and expression of AEG-1 in relation to clinical outcome and radiotherapy response in colorectal cancer patients and cell lines

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Background: Colorectal cancer (CRC) is the third most common cancer in men and the second in woman worldwide. The therapy of CRC has been critically improved during the past two decades, but the treatment response varies significantly between different treatments and patients. Therefore it is of need to search for biomarkers for more suitable prognosis and treatment. The aim of this study was to investigate genetic variants of the astrocyte elevated gene-1 (AEG-1), its expression in CRC patient samples and colon cancer cell lines and the potential correlation to clinicopathological variables and treatment response.

Materials and Methods: We analysed genetic variants and the mRNA and protein expression of AEG-1 in 593 CRC patient samples by direct Sanger sequencing, qPCR and immunohistochemistry. Of the patients, 158 rectal cancer patients were enrolled in the Swedish clinical trial of preoperative radiotherapy (RT). AEG-1 expression in response to γ-irradiation was analysed by Western blot in 5 colon cancer cell lines. We inhibited the AEG-1 expression by siRNA in the cell lines, and their survival was analysed after γ-irradiation. siRNA in the cell lines, and their survival was analysed after γ-irradiation.

Results: By direct sequencing we found 29 variants, of which 12 were novel. Six of the variants were exonic and 23 intronic. The variant c.1353G>A, rs2331652 was found only in 4 samples and the variant c.1679-6 T>C in only one sample. In the cell lines KM12C, KM12SM and KM12L4a we found a deletion in exon 4 (c.595_598delAGAG). AEG-1 mRNA and protein expression revealed a significant increased AEG-1 expression in the primary tumors and metastases compared to the normal mucosa in all patient cohorts ($p<0.006$) and on the mRNA level a higher expression in tumors located in the rectum compared to tumors in the colon ($p=0.047$). In the rectal cancer patients from the Swedish trail of preoperative RT, a high AEG-1 expression correlated with a higher risk of distant recurrence and disease free survival ($p=0.001$ respectively) independently of the tumor stage, only in patients receiving preoperative RT. AEG-1 knock-down in the radioresistant cell lines

KM12L4a, SW480 and SW620 resulted in reduction of the survival after radiation, but not in the radiosensitive cell lines KM12C and HCT116. The AEG-1 expression was up-regulated shortly after 2 Gy γ-radiation in KM12C, KM12L4a and SW480, but not in SW620 and HCT116.

Conclusion: We found several novel AEG-1 variants. The AEG-1 expression at the mRNA and/or protein level was up-regulated in the primary tumour and metastases compared to the normal mucosa. Furthermore we showed for the first time that the AEG-1 expression is an independent prognostic factor for distant recurrence and disease-free survival in rectal cancer patients after treatment with preoperative RT. The cell line studies suggest AEG-1 as a promising radiosensitizing target for rectal cancer.