Clinical Cooperation Unit Radiation Oncology (E0500 / E050)

Head: Priv. Doz. Dr. med. Dr. rer. nat. Jürgen Debus

Senior Scientists
Priv. Doz. Dr. Peter Huber
Dr. Jürgen Jenne
Dr. Peter Peschke
Dr. Daniela Schulz-Ertner*
Dr. Christoph Thilmann
Prof. Dr. Ivan Zuna

Scientists
Dr. Klaus Braun (4/00-)
Dr. Heike Corban-Wilhelm (6/01-)
Bernd Didinger
Dr. Karin Henke-Wendt
Dr. Klaus Herfarth*
Dr. Holger Hof*
Dr. M. Kissel* (-7/02)
Dr. Marc Münter*
Gregor Remmert*
Dr. Angelika Zabel*

Postgraduate and Graduate Students
Amir Abdollahi
Gabriela Divkovic (4/02-)
Ping Gong
Sandra Hessenthaler
Twan Lammers (9/02-)
Sylvia Münter
Daniel Poerschke
Alexandra Roth
Mario Steinbach
Lennart Thilmann
Fatemeh Vorodi
Heike Zieber

Technicians
Marion Bachmann
Dietmar Greulich
Katja Kuhn (5/02-)*
Rainer Kühnlein
Tobias Richter (-12/01)
Alexandra Tietz

Secretary
Ingrid Reinke (5/01-)
Renate Haselmann

Civil Servants
Michael Thiemer (7/01-4/02)
Daniel Burns (5/02-)
Christoph Guggenberger (8/01-5/02)

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Scientific concept of the Clinical Cooperation Unit Radiation Oncology
The aim is the development of new radiooncological strategies. Over the past two years substantial contributions to the radiooncological treatment of patients were made. This includes not only improved technological approaches to treatment delivery but also the development of biologically based optimization of radiation effects. These tasks were performed in very close co-operations with the Department of Medical Physics Prof. W. Schlegel and the other departments of the research program. Due to a close co-operation with the Department of Radiation Oncology at the University Hospital of Heidelberg it was possible to conduct clinical phase I, I/II and phase III trials which examine safety and reliability of recently established therapeutic methods and planning procedures. The optimization of computerized radiotherapy planning and simulation may hereby provide substantial improvement of the results. Carbon ion radiotherapy has an increased radiobiological effectivity and allows precise dose deposition. In the frame work for a collaborative study we are investigating the application of heavy ion therapy in patient system base of skull tumors. On the experimental level, it is our aim to examine if the individual radiosensitivity can be predicted by molecular biological approaches and also eventually therapeutically controlled. Moreover, new approaches with non-invasive therapeutic methods for example the use of high intensive ultrasound therapy in the treatment of tumors is evaluated. The ultimate goal of the clinical co-operation unit is to enhance translational research in the area of radiation oncology.
Conformal Radiotherapy Using Intensity Modulated Beams

The goal in radiotherapy is to achieve local tumor control without exceeding tolerance doses of the surrounding radiosensitive normal tissue. New conformal techniques in radiotherapy try to adapt dose distribution as closely as possible to the target volume. This could be realized using multiple portals with individual beam shaping. However, there are cases of complex shaped tumors in close relationship to organs at risk where no sufficient dose distribution could be achieved with conventional technique. This could be seen especially in concave shaped targets enclosing organs at risk. An improvement in dose distribution could be achieved using diverse intensities within each portal. Thus, dose escalation to the tumor without increased complication rate seems possible in several cases.

In the past efforts were taken to develop utilities for radiotherapy planning, delivery and verification of intensity modulated beams. An inverse planning software (KonRad®) was established to check the difference between applied and desired treatment plan and if necessary to correct the applied plan.

The clinical feasibility of IMRT is tested for different tumor sites in the phase II study ‘Conformal Radiotherapy with Intensity Modulation’. First patients included in the study were patients with complex shaped meningiomas of the skull base. Until now 80 patients were treated in cases in which a satisfying dose distribution was not achievable with conventional treatment techniques.

In total 350 patients were treated with IMRT at the DKFZ with the following diseases:

- base of skull tumors
- tumors of the head and neck
- paraspinal metastases and tumors
- mesothelioma
- prostate cancer
- breast cancer after breast conserving surgery

For these indications the use of IMRT is feasible and safe in the tumor sites mentioned above with the fixation devices available in our department. IMRT may prove to be a valuable therapeutic modality for complex shaped target volumes adjacent to critical structures.

Here, a special focus is the improvement of radiotherapy of breast cancer. The aim is implementation of IMRT into clinical routine of breast cancer. Because of medial tumor site, the parasternal lymph nodes were included into the target volume. In these cases improvement of local control and reduction of side effects can be expected. A phase III study is planned for this subgroup. Additionally, IMRT enables to introduce new principles into radiotherapy. An integrated boost as a new treatment concept delivers a high dose to the macroscopic tumor site and simultaneously a homogeneous dose to the surrounding tissue of microscopic spread. In a treatment planning study for malignant gliomas we could demonstrate the superiority of an integrated boost based on inverse planned IMRT over forward planned SCRT. To compare the effectiveness of this treatment procedure with conventional treatment of patients with high grade gliomas a phase III trial is initiated.

Fig. 1: High-dose irradiation of a prostate carcinoma using five intensity-modulated 15 MV photon fields (100% = 76 Gy)

Fig. 2: Radiation treatment of a patient with a funnel chest. Internal mammary chain was included into the target volume (a) Dose distribution of a conventional treatment using two tangential wedged 6 MV photon beams combined with 15 MeV electrons; (b) IMRT (12 intensity modulated 6 MV photon beams)

Stereotactic Radiation Therapy of Cranial and Extracranial Targets

In cooperation with: Div. of Medical Physics (Prof. Dr. W. Schlegel); Div. of Oncological Diagnostic and Therapy (Prof. Dr. G. van Kaick) DKFZ

Stereotactic radiation therapy has the potential of delivering a high radiation dose to a defined tumor volume with a steep dose gradient to the surrounding normal tissue. We
have evaluated the use of stereotactic radiation in several tumor entities. Some of the results are discussed more in detail:

1. Acoustic neuroma
Acoustic neuromas were traditionally treated by surgical resection. However, fractionated stereotactic radiation therapy might be an alternative to the traditional approach. Our results are superior to published neurosurgical series and to radiation series using the gamma knife if tumor control and side effects are taken into account. The results show that fractionated stereotactic radiation therapy might have a major role in the treatment of acoustic neuromas.

2. Meningeomas of the skull base
A complete surgical resection of meningeomas of the skull base is often not possible. This is due to the close neighborhood of major blood vessels and the optic nerves. Fractionated stereotactic radiation therapy might be the treatment of choice for patients with this kind of tumor. Our data show the effectiveness of fractionated stereotactic radiation treatment of skull base meningeomas. Comparison of our results with other techniques underlines the potential of this treatment compared to neurosurgery with or without conventional radiotherapy.

3. Chordomas
A typical location for chordomas is the clivus in the skull base. Since these tumors are relatively radioresistant, high radiation doses have to be applied for sufficient tumor control. However, using conventional techniques, major side effects are likely due to the neighborhood of radiosensitive brainparts. Using proton or heavy ion radiation therapy, local tumor control rates of 60% can be achieved. However, these techniques are expensive and limited to certain regions in the world. Using fractionated stereotactic radiation therapy, chordomas can safely be treated with only minor decrease in local tumor control compared to ion therapy. However, we hope to improve these results further using dose escalation with carbon ion therapy.

4. Liver tumors
Several more or less invasive local techniques are available for inoperable liver tumors. We have evaluated a non-invasive technique for the treatment of these tumors using stereotactic single-dose radiation therapy. The results showed any major side effects. Local failures were mainly due to low dose (low dose total or low dose at the tumor margins). Patient survival depended significantly on the presence of other extrahepatic systemic metastases at the time of treatment. Our results show that stereotactic single-dose radiation therapy is a feasible, effective and safe method for the treatment of inoperable liver tumors if the tumors are limited in size and not directly adjacent to bowel parts.

5. Prostate cancer
Improvements in local tumor control of prostate cancer can be achieved if the applied radiation dose is escalated. However, dose escalation is limited if nearby radiosensitive organs like bladder or rectum cannot sufficiently spared from the high dose region. Intensity modulated therapy (IMRT) might be the answer for further dose escalation, if an accurate and reproducible positioning of the patient allows closer safety margins around the prostate.

We have evaluated the self-developed body mask, which is normally used for the treatment of paraspinal tumors, for the accuracy in the IMRT treatment of prostate cancer. The mean variations of the bony structures varied by only 0.9 mm in anterior-posterior direction and 0.2 mm in latero-lateral direction. The mean variations of the prostate could be limited to 1.7 mm in anterior-posterior direction and 0.2 mm in latero-lateral direction. This positioning accuracy allows a highly precision radiation therapy with closer safety margins than in conventional 3D planned radiation therapy.

Carbon Ion Radiotherapy
D. Schulz-Ertner, C. Thilmann, J. Debus
In cooperation with: Prof. M. Wannenmacher, Dept. of Radiation Oncology, University of Heidelberg; Prof. G. van Kaick, Div. of Oncol. Diagnostics and Therapy, Prof. W. Schlegel, Dr. O. Jäkel, Dr. C. Karger, Div. of Medical Physics, DKFZ; Prof. G. Kraft, Dr. T. Haberer, GSI Darmstadt; Dr. W. Enghardt, FZ Rossendorf.
The Division of Radiation Oncology started carbon ion radiotherapy at the Gesellschaft für Schwerionenforschung (GSI) Darmstadt within a feasibility study in 1997. Before patient treatments started a radiation unit was built at the heavy ion synchrotron of the GSI and major future directed technical and radiobiological innovations have been implemented. For the first time, tumor conform application of carbon beams was realized by intensity-controlled raster scanning with pulse-to-pulse energy variation [Haberer T et al. Nucl Inst Phys Res 1993; 330; 296-305]. All patients had 3-dimensional treatment planning including a biological plan optimization using the treatment planning program TRIP [Scholz M et al. Int J Radiat Oncol Biol Phys 1994; 66; 59-75]. A PET camera is used for online beam verification.
The study contained patients with chordomas and low grade chondrosarcomas of the skull base, adenoid cystic carcinomas malignant meningeomas and other tumors. These tumors are known to be relatively radioresistant against conventional photon irradiation. Proton radiotherapy has been shown to improve outcome in chordomas and low grade chondrosarcomas but its availability is limited. In adenoid cystic carcinomas radiotherapy with heavy particles as neutrons results in improved local control rates compared to photon irradiation but causes
severe side effects. Malignant meningiomas commonly recur within the former irradiated fields even after high tumor doses. Carbon ion therapy presents a promising therapy option in the management of these tumors.

**Experimental Strategies**

P. Huber, J. Jenne, P. Peschke, I. Zuna, K. Braun, H. Corban-Wilhelm, R. Rastert, I. Simiantonakis

In cooperation with: Siemens AG, Medizinische Technik, Erlangen; EDAP/Technomed Lyon, France; J. Víba, Dept. of Electromagnetic Field, Czech Technical University Prague, Czech Republic; L. Poušek, Centre for BioMedical Engineering, Czech Technical University Prague, Czech Republic

**Ultrasound therapy and first patient treatment with MRI-guided focused ultrasound**

The goal, to treat a patient with invasive breast cancer with a new non-invasive image guided therapy modality, has been achieved. Ultrasound as a form of mechanical wave transmission is widely used in almost all medical specialties, mainly for diagnostic purposes. However, interactions of ultrasound with biological tissues are dependent on the acoustic parameters such as peak pressure amplitude and intensity. Increasing pressure and intensity enables either the thermal or the non-thermal therapeutic capabilities. Both therapeutic approaches are characterized by an excellent focusability combined with a high penetration depth, allowing extensive energy densities in deep tissue layers while sparing intermediate tissues. Non-thermal ultrasound shock wave applications have been proven to be successful in clinical lithotripsy, especially for the removal of kidney stones. The side effects in clinical lithotripsy such as local hemorrhage and edema have prompted research to investigate pulsed high-energy ultrasound shock waves (PHEUS) for the treatment of experimental tumors. PHEUS has been shown to cause cytotoxic effects on tumor cells in vitro and in vivo, and to induce rapid onset of ischemia in experimental tumors with subsequent necroses in tumors leading to tumor growth delay. The thermal effects of ultrasound are induced by continuous wave high-intensity focused ultrasound (HIFU) allowing for exact temperature distribution inside the body. HIFU penetrates well through soft tissue and can be focused through the intact skin to volumes with dimensions of a few millimeters. The energy absorption in tissue can induce temperature elevations of 70°C to 90°C in the focal spot within a few seconds that instantaneously denaturates protein structures. Because of the sharp thermal gradients the boundaries of the treated volume are sharply demarcated without damage of the overlying or adjacent tissues. In combination with temperature sensitive magnetic resonance imaging (MRI), HIFU therefore is a promising tool for local tumor therapy in all ultrasound accessible sites such as breast, prostate, liver, kidney, head and neck. The major goal of the research was to develop a MRI guided HIFU treatment unit that could be used for the therapy of breast cancer. To this end, experimental and finally a clinical treatment unit was developed. The clinical therapy unit consists of a MRI compatible ultrasound applicator including the ultrasound source, a positioning system and a specific MRI coil to optimize imaging of the focal region. Further elements are a supply unit and a workstation controlling the whole unit.

The applicator is built up as a bowl with a pot-shaped deepening to accommodate the breast for treatment. In the deepening an ultrasound transparent window allows coupling the ultrasound field into the breast tissue. The ultrasound transducer has a focal length of about 70 mm and operates at a center frequency of 1.7 MHz. To treat the target region the sound source is moved by three linear actuators. These extensible links consist of hydraulically driven linear stepper motors (resolution <0.1 mm), supplied by pressure pulses. Fiber optic light barriers controlling every single step and reference positions control positioning of the system. Several sensors control the function of the system and guarantee a safe operation.

The supply unit is separated from the MR scanner and provides the ultrasound application inside the MR magnet. The whole therapy system is software controlled. Special software enables therapy planning including segmentation of the target region on MRI planning images and calculation of the therapy plan. For therapy control MR temperature images were analyzed and visualized on the control workstation.

For therapy the patient lies in prone position with the breast fixed in the deepening of the applicator. The applicator bowl itself is fixed on the table of a conventional 1.5 T MR scanner (Magnetom Vision Plus, Siemens AG) and can be fixed in several positions to adjust it near to the target volume, e.g. a tumor, inside the breast.

We could demonstrate that breast cancer in a human patient can non-invasively be treated in a single session through the intact skin by a combination of focused ultrasound and MRI guidance with interactive target segmentation and temperature control. Due to the immediate effect without damage to the surrounding healthy breast tissue, the absence of any anesthesia and scars, MRI-guided non-invasive focused ultrasound therapy has potential for breast cancer therapy, and other oncologic entities.

**HIFU treatment acceleration by modification of sound field and sonication modalities**

One drawback of the new therapy method is the rather long treatment time. It is a result of the ultrasound focus volume (≈mm³) and the relatively large tumor size (≈cm³). A lot of ultrasound shots have to be applied to coagulate the whole tumor (for bigger targets more than one or two hundred). To overcome this limitation different methods for reducing the treatment time were investigated.

One possibility for acceleration of a treatment is to reduce the pause time after each shot. The pause is necessary to avoid overheating and after a 9 s pulse we have to wait 50 s until the temperature rise has disappeared. Computer simulations showed that with an optimized ordering of the single pulses the pause time could be reduced to 13 s without tissue overheating. This sonication method was tested in animal experiments where we sonicated the thigh muscle of a rabbit. It was possible to induce coherent lesions in the muscle and there was no increase in side effects compared to the normal treatment.
We developed also special lenses for our ultrasound system and tested them. For finding the lens suited best for our purpose, the sound fields for different lens geometries were simulated and compared to each other. The lenses that broadened the focal spot most and avoided a higher length were then experimentally tested and their sound fields were measured. We found that mode-n lenses fulfill the requirements best. Mode-n lenses are asymmetric lenses which induce a phase shift \( f(\Theta) = n \Theta \). The mode-1 lens proved to be especially suitable. It enlarges the focus diameter by a factor of two without increasing its length, thereby reducing the treatment time by a factor of 4. The mode-1 lens was also tested in animal trials and we were able to create a coherent lesion without side effects.

**Noninvasive temperature monitoring using ultrasound tissue characterization method**

At present, invasive thermometer probes are clinically used for temperature monitoring during thermotherapy. Such measurements suffer from the limited number of localized measuring points, the lack of spatial resolution and often represents due to its invasive character a burden for patients. The ultimate goal of this research activity was to develop a non-invasive procedure for detecting temperature distributions in thermal treated tissues. A new computer – based system has been developed and tested in an experimental study. It consists of an intelligent regulation loop controlling high frequency microwave generator according to the maximal measured temperature in the tissue and a special microwave applicator. The loop is also equipped with an invasive thermometer. During the hyperthermia treatment series of ultrasound B-mode images using the Acuson Sequoia 512 diagnostic ultrasound system were obtained. Several texture parameters were evaluated from the obtained ultrasound images. These parameters were correlated with the invasively measured temperature during the therapy session. For 60 rat samples a strong correlation between the mean grey level in the selected region of interest in the ultrasound pictures and the invasively measured temperature in the range 37-44°C was found. The correlation coefficient between the mean grey level and the invasively measured temperature was 0.96 ± 0.05. The dependency of the evaluated temperature on the normalized mean grey level was a linear function, with a start point at 37.00 ± 1.18 and slope equal 0.94 ± 0.23. The next best parameter was the mean gradient. The correlation of the mean gradient and invasively measured temperature was \( r = 0.74 ± 0.21 \). All other parameters did not show any higher correlation with the temperature. To get information about the temperature distribution in the whole scanned tumor area, every image of data series was divided in equal sub-regions (12 × 12 pixels) and on each region the mean grey value was evaluated. The matrix of results was used for pseudo-color coding of greyscale B-mode images.

The use of ultrasound for non-invasive thermometry possesses several benefits. The approach assures sufficient spatial resolution for MT purposes (vs. dielectric properties measurement), the monitoring of temperature distribution is possible in a much more extensive area than for invasive techniques. The ultrasound penetrates the tissue in sufficient depth (vs. infrared techniques) and represents no burden to a patient (vs. X-CT). The purchase and running costs are relatively low (vs. MR) and there is no problem to transport the ultrasound equipment to a patient or to other therapy equipment (vs. MR). On the other hand, there are some limitations regarding gas and bone areas within monitored regions.

**Molecular Transport**

Recent progress in biotechnology and peptide synthetic chemistry have resulted in the large-scale production of molecules which are of outstanding interest for structure-functional studies in basic molecular biology and may gain importance as potential therapeutics in future. Among these, peptide nucleic acids (PNAs), which represent synthetic, non-ionic DNA mimics gained attraction due to their favorable physico chemical and biological characteristics. PNAs are resistant to nuclease and protease digestion and hybridize to complementary sequences with higher affinity than analogous DNA oligomers. Poor cellular import of PNAs is the major limiting factor and hence procedures for delivery of these compounds into cells are urgently needed. To facilitate nuclear delivery of biomolecules we developed and synthesized a modular transporter bearing a cellular membrane transport peptide and, as a cargo a 16-mer peptide nucleic acid (PNA) covalently linked to a nuclear localization signal (NLS[SV40-T]). Transport peptide and PNA are connected via N-terminal activated cysteine to form degradable disulfide bonds. Internalization and subsequent delivery of PNA to the nucleus was verified in living and fixed cells by Confocal Laser Scanning Microscopy (CLSM) and Fluorescence Correlation Spectroscopy (FCS). Double-labeling experiments indicate the cytoplasmic cleavage of the two modules and the effective nuclear import of the chromophore-tagged cargo. A non-degradable linker between transport module and cargo as well as a construct without NLS did not enable nuclear PNA import under the described experimental conditions. FCS-measurements revealed that most of the PNAs delivered into the cytoplasm by the modular transporter are anchored or encapsulated, indicating that intracellular transport of these compounds is not governed by molecular diffusion. Our results clearly demonstrate efficient compartment-directed transport using a synthetic, non-toxic modular transporter in living cells.

**Suicide Gene Therapy: Failure of the Bystander Effect**

Enzyme/prodrug systems are used to localize the toxic effects of drugs to tumor cells. This involves gene transfer of a viral or bacterial enzyme into tumor cells, which then converts an inactive form of a drug ("prodrug") into a toxic metabolite, leading to tumor-cell death. Prodrugs even at high concentrations, are non-toxic unless specifically activated by cellular conditions or enzyme to toxic metabolites. The most studied enzyme/prodrug combinations are the *Herpes simplex* virus type-1 (HSV) thymidine kinase (TK) and the bacterial enzyme cytosine deaminase (CD) from *E. coli*. One of the key aspects of suicide gene/prodrug strategies is the reliance on the so-called bystander...
**effect**, which can compensate for the inability to achieve transfection and expression of the suicide gene in 100% of the tumor cells *in vivo*; without this effect, nonexpressing cells will survive drug therapy and cause tumor regrowth. In our case, toxic metabolites generated in an "expressing" cell can be transferred to a "nonexpressing" cell via gap junction intercellular communication (GJIC) (TK/GCV System) and via an *extracellular* route involving active or diffusional export of the 5-FU generated by CD.

The rat prostate tumor cell line R3327 AT-1 was trans- fected with a gene coding for a fusion protein comprised of cytosine deaminase (CD from *E. coli*) and thymidine kinase (TK from *Herpes simplex virus*, HSV-1). The resulting AT-1/CDglyTK cell line was sensitive to the prodrug 5-fluo- rocytosine (IC_{50} = 78 µM, 96-h incubation) via CD and to ganciclovir (GCV, IC_{50} = 1 µM, 96 h) via TK. Subcutaneous tumors generated from 100% CDglyTK+ cells responded well to 5-FC therapy (500 mg/kg, i.p., 14 daily treatments, 4/7 animals in remission) and to GCV therapy (30 mg/kg, i.p., 14 daily treatments, 5/6 animals in remission). However, experiments with mixtures of CDglyTK+ and CDglyTK- cells showed low levels of connexins (intercellular gap junctions) and no bystander effect for nontrans- fected cells using either 5-FC or GCV therapy. Further- more, experiments with mixtures of CDglyTK+ and CDglyTK- cells showed low levels of connexins (intercellular gap junctions) and no bystander effect for nontrans- fected cells using either 5-FC or GCV therapy. Furthermore, ^19F-NMR spectroscopy showed that incubation of cultured CDglyTK+ cells with 774 µM 5-FC for 16 h re- sulted in the following intracellular concentrations: 5-FC = 314 µM, 5-FU = 52 µM, cytotoxic fluoronucleotides = 163 µM, extracellular 5-FU reached only 6.4 µM. Thus, in this model system intracellular trapping of 5-FU (slow export) contributes to the failure of the CD/5-FC bystander effect via an extracellular route.

**Carrier-bound Radiosensitizers**

Inadequate delivery is the single most important factor de- laying optimized application of both conventional low mo- lecular weight anti-cancer drugs and advanced molecular medicines. The challenge to drug delivery systems is to find ways to stabilize the therapeutic molecules by in- creasing their circulation life time and to deliver them se- lectively to their target sites. The ultimate goal of the present research activity is to exploit the physiological characteristics and transport properties of synthetic poly- mers with special reference to therapeutic agents pos- sessing radiosensitizing properties.

More than 60% of cancer patients in the US receive radio- therapy with curative or palliative intent. About 30% of the patients with localized disease eventually succumb to the disease, and would stand to benefit from improvements in radiotherapy. Although combined treatment modalities, e.g. chemotherapy and radiotherapy, have produced sig- nificantly enhanced response and survival rates in clinical trials, the limitations of current therapy modalities provide a compelling rationale to develop alternative strategies for the targeted delivery of therapeutics into solid tumors. An approach to circumvent these problems is to develop tech- niques which favor tumor-directed transport. Presently, two major strategies are being investigated to achieve site- specific drug delivery involve: (a) active targeting to ligands with selective affinity for biomolecules (antibodies, receptors, carbohydrate moieties), and (b) passive target- ing systems utilizing the unique vasculature of solid tu- mors. Since it has been shown that macromolecules with a long circulating half-life are deposited preferentially in solid tumors, originally referred to as the enhanced perme- ability and retention effect (EPR), interest developed in us- ing liposomes, nanoparticles, and soluble macromol- ecules, obtained either from endogenous proteins, e.g. hu- man serum albumin (HSA) or synthetic polymers, e.g. poly((N-2-hydroxypropyl)-methacrylamide) (HPMA), as car- rier compounds. Although the underlying biological mechanism of the EPR-effect is still poorly understood, tu- mor accumulation of long-circulating macromolecules is considered to be a consequence of structural and func- tional characteristics of the tumor vasculature, as well as the lack of effective lymphatic drainage.

The synthetic carrier molecule pHMA is an inert, un- charged polymer which exhibits little immunogenicity and high physicochemical flexibility. In basic studies the trans- port characteristics of HPMA were examined *in vivo* with respect to clearance, half-life and biodistribution using dynamic scintigraphy and dissection analysis. To optimize tumor-directed delivery, it was necessary to evaluate the influence of molecular weight and molecular charge of polymers on tumor and organ distribution. Intravenously administered pHMA showed a molecular weight-depen- dent plasma circulation time leading to an enhanced EPR- effect with a continuous uptake to ~16 % of the applied initial radioactivity in an anaplastic prostate tumor model. Contrary to the tumor, most normal tissues are being cleared faster after showing an initial uptake of pHMA. To determine the topohistological distribution of HPMA in normal tissues and tumor compartments, we developed a cellular localization technique using biotin labeled pHMA.

dFdCyd has been suggested to optimize treatment. A
promising alternative strategy to increase the therapeutic ratio may be to improve drug selectivity via polymeric carrier systems. Several pHMPA-dFdCyd conjugate derivatives were synthesized to test our hypothesis that a concurrent radio-chemotherapy can be optimized, if dFdCyd is locally delivered to the tumor while sparing surrounding normal tissues. Currently, two types of conjugates differing in structure and mode of drug release are under investigation with special emphasis on plasma circulation time, EPR-effect and organ distribution.

Vascular Biology, Angiogenesis and Radiotherapy

In recent decades, radiation research has concentrated primarily on the cancer cell compartment. We demonstrate that ionizing radiation is a potent angiogenic agent that inhibits endothelial cell survival, proliferation, tube formation and invasion. VEGF and bFGF were able to reduce the radiosensitivity of endothelial cells. Yet, it is also found that radiation induces angiogenic factor production by tumor cells that can be abrogated by the addition of anti-angiogenic agents. Receptor tyrosine kinase inhibitors of Flk-1/KDR/VEGFR2, FGFR1 and PDGFR, inhibit endothelial cell survival, proliferation, tube formation and migration in an in vitro system. In a co-culture system of PC3 prostate cancer cells and endothelial cells, irradiation of the PC3 cells enhanced endothelial cell invasiveness through a Matrigel matrix, which was inhibited by SU5416 and SU6668. Furthermore, ionizing radiation upregulated VEGF and bFGF in PC3 cells and VEGF in endothelial cells. Together these findings suggest a radiation-inducible protective role for tumor cells in the support of their associated vasculature that may be downregulated by co-administration of angiogenesis inhibitors. In vivo we found that SU5416 and SU6668 increased effectiveness of fractionated radiation. Therefore our results rationalize concurrent administration of angiogenesis inhibitors and radiotherapy in cancer treatment.

We found that the combination of a “direct” angiogenesis inhibitor (endostatin) with an “indirect” anti-angiogenic compound such as SU5416 (VEGFR2 RTK-inhibitor) substantially reduces endothelial proliferation, survival, migration/invasion and tube formation. In vivo, the combination of SU5416 and low-dose endostatin reduced tumor growth in human prostate (PC3), lung (A459) and glioma (U87) xenograft models. These angiogenic agents were more effective in combination than when used alone. Similarly, this combination therapy markedly reduced functional microvessel density, tumor microcirculation and blood perfusion as detected by intravital microscopy and contrast-enhanced Doppler ultrasound.

Expression Profiling of Endothelial Cells treated with Radiotherapy, Hypoxia, or Endostatin

One of the major goals to better understand and eventually modulate radiation effects is the identification of involved genes. We used a large DNA-Chip to get expression profiles from complete human Unigene II clusters which contain 74,834 elements covering almost 95% of the human genome. In order to determine the genes involved in early response to radiation in endothelial cells, the total RNA in human microvascular lung endothelial cells was isolated 4 h after 2 Gy radiation. RNA quality was tested using the 28S vs. 18S ratio with lab on chip technology. Hybridization was 2-fold for each spot with 4 independent measurements. We identified a series of genes in human microvascular lung endothelial cells whose expressions varied in response to 2 Gy radiation in vitro, many of which were confirmed using quantitative real-time RT-PCR. 928 genes were up-regulated more than 2-fold, and 504 genes were down-regulated more than 0.5-fold. Among the regulated genes were in particularly many genes known to be related to the coagulation cascade including PAR2, FGB, F12, F11, the inflammation cascade including ILs, ITGB1, ITGB8, TRAFs, and related to angiogenesis including VEGF, VEGFR, EGFR, STATs and many others. Other regulated genes were genes involved in cell cycle (>30 genes), proteases (>40), suppressor genes (>10), and cancer in a universal sense (>100). Detecting alterations in gene expression associated with radiation should be a way to uncover clues as to the specific molecular changes that contribute to the behavior of microvascular endothelial cells in response to radiation. Our findings demonstrate that radiation can affect the expression pattern of microvascular endothelial cells, and that genes specifically involved in coagulation, inflammation and angiogenic pathways may play important roles in the acute response to this stress.

Endostatin, an endogenous angiogenesis inhibitor, promotes apoptosis and inhibits migration, invasion and proliferation of endothelial cells. Using genome-wide expression profiling covering 95% of the human genome, coupled with RT-PCR and phosphorylation analysis on antibody arrays, we demonstrate that endostatin downregulates many signaling pathways in human microvascular endothelium previously associated with pro-angiogenic activity. Additionally, endostatin is found to upregulate many anti-angiogenic genes. It is here demonstrated for the first time that the set of gene expressions underlying the angiogenic balance in tissues can be molecularly reset en masse by a single protein. These results point to a pervasive and entrenched role for endostatin in the regulation of endothelium. Profiling further reveals the regulation of well-characterized genes not heretofore associated with angiogenesis. Together, these complex inter-pathway communications triggered by endostatin comprise an intricate signaling network activated in endothelium that both recapitulates and extends on current understanding of the angiogenic process.

In addition, we identified a series of genes and proteins whose expressions varied with different exposure times under hypoxic conditions in vitro. Hypoxia-regulated genes are involved in different pathways like endothelial cell-leukocytes interactions, expression of vasoactive genes, tissue remodeling, tissue proteases and transcription factors. Several pathways such as Id1, Ephrins and Stats were found to be potentially involved in endothelial cells hypoxic signaling. Finally, the expression profiling data results in
the identification of several potentially novel uncharac-
terized genes (HIGS) that were induced by hypoxia. Pro-
tein chips (Ciphergen®) were used to investigate differen-
tially-expressed proteins. We detected 18 proteins, which are
up- or down-regulated depending on the length of hy-
poxic exposure, their hydrophobic (H4), anionic (SAX) and
cationic (WCX) statuses, or the ability to bind on ions
(IMAC). Detecting alterations in gene expression associ-
ated with radiotherapy, hypoxia, endostatin and other rel-
evant stimuli should evolve as an efficient way to uncover
key players of specific molecular changes in microvascu-
lar endothelial cells.

Publications (* = external co-author)

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