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# Molecular imaging and photodynamic therapy in hepatoblastoma

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## 1. ABSTRACT

Molecular imaging is a novel field in cancer research combining various in vivo imaging modalities with molecular biology. Different techniques such as magnetic resonance tomography (MRI), positron emission tomography (PET), optical imaging methods (bioluminescence, fluorescence), or combination of these are used in basic research as well as in patients in different tumor entities. In hepatoblastoma (HB), there are only few reports on molecular imaging methods in a preclinical (optical imaging) and clinical setting (PET, PET-CT). Unimprovable treatment outcomes of patients in advanced tumor stages require novel treatment approaches. Photodynamic diagnosis (PDD) and photodynamic therapy (PDT) are novel diagnostic and therapeutic tools. Photodynamic diagnosis allows in vitro and in vivo detection of tumor cells using their fluorescending behaviour. PDT is a novel anticancer treatment approach leading to tumor cell destruction via apoptosis. In hepatoblastoma, there are only few reports on in vitro and in vivo studies using this treatment modality. First results seem to be promising and further studies will be required to further evaluate these techniques and to transfer them into clinical settings. This paper reviews different modalities of molecular imaging, photodynamic diagnosis and photodynamic therapy in childhood hepatoblastoma.

## 2. INTRODUCTION

Treatment of childhood hepatoblastoma (HB) has been improved remarkably in the past mainly by the establishment of international treatment optimizing studies. A combination of chemotherapy and surgical tumor resection or liver transplantation is used in this tumor entity. In standard tumors even preoperative treatment reduction was successfully applied (1). Besides these efforts, there are still known unsolved problems especially in advanced tumor stages, relapsed or metastasized tumors. Over recent years, there was no major improvement of outcomes in these complex conditions. Therefore, novel diagnostic and therapeutic approaches need to be evaluated in order to improve the survival of affected patients.

Molecular imaging is a growing field in cancer research combining molecular biology and in vivo imaging. Imaging modalities consist of magnetic resonance imaging (MRI), computed tomography (CT), optical imaging using various methods such as fluorescence or bioluminescence, as well as positron emission tomography (PET) or combinations of these techniques. The goal of molecular imaging is to investigate biological processes within the body. In cancer research, the main focus of molecular imaging is the visualization of cancer cell trafficking, tumor-cell interactions, tumor-host interactions,

development and behavior of metastases, as well as visualization of metabolic pathways.

Photodynamic therapy (PDT) is an alternative anticancer treatment approach based on the administration of a non- or weakly toxic photosensitizer. The success of PDT depends on the effectiveness of the delivery of the photosensitizer to the target tissue. If an adequate uptake of the photosensitizer occurs in the tumor cells, exposure with light of the appropriate wave length causes production of singlet oxygen, which causes apoptotic cell death (2,3).

Up to now, there are only few reports on molecular imaging and photodynamic therapy in childhood hepatoblastoma. The aim of this paper is to review molecular imaging and photodynamic approaches in hepatoblastoma based on their evaluation and to clarify the role of these approaches in other pediatric solid tumors.

## 3. MOLECULAR IMAGING

Molecular imaging combines in vivo imaging and molecular biology using techniques such as optical imaging, MRI, CT or PET, or combinations of those.

### 3.1. Optical imaging – Fluorescending proteins

Optical imaging aims to visualize tumor cells in the living organism. Therefore, tumor cells need to be labeled with a specific marker, which allows the detection of these cells in vitro or in vivo. Among these markers, fluorescending proteins play an important role for in vitro and in vivo visualization studies. Tumor cells can be labeled with fluorescending proteins after transfection of a specific vector encoding the appropriate protein (4). After excitation with light of the appropriate wavelength, tumor cells can be detected by their fluorescending behaviour. The first described fluorescending protein was green fluorescent protein (eGFP), which was used to analyze myofibrillogenesis in living cells as well as to study treatment procedures in cell lines (5,6). In animal models of cancer, fluorescending proteins have enabled the visualization of tumors and metastases in orthotopic localization (7). GFP has been used for the detection of the primary tumor growth of pancreatic cancer and its metastases (8). Real time tracking of lung metastases of fibrosarcomas as well as visualization of bone and lung metastases of human breast cancer cells was feasible in previous studies using GFP (9-11). In animals with bladder cancer, GFP-expressing cells could be detected in the voided urine and this could serve as a novel diagnostic tool in this tumor entity (12).

Besides GFP, other fluorescending proteins like red fluorescending protein (DsRed) have been developed. The main advantages of DsRed are the longer wavelength and the lower energy emission. Therefore, DsRed transmits more efficiently through whole tissue compared to GFP and is better distinguishable from background fluorescence (13). The authors used DsRed for in vivo visualization of pediatric rhabdomyosarcoma xenografts as well as for in vivo detection of rhabdomyosarcoma metastases (4,14). Additionally, our group was able to demonstrate cancer cell trafficking using DsRed in childhood rhabdomyosarcoma. The results of these studies showed that metastatic invasion of RMS is based on tumor cell migration, accumulation and extravasation (14).

In hepatoblastoma, there is only one report of the usage of fluorescending proteins. Warmann et al. reported on a successful transfection of human hepatoblastoma cells with GFP. For the first time, they were able to establish a stable GFP-transfected HB cell line after liposomal vector transfection, usage of a selection antibiotic (G418) and FACS-selection (15). Transfection rates of HB cells were only 15% which were lower compared to other malignancies. The problem of the usage of hepatoblastoma cells is the fact that they are difficult to culture and transfection with a fluorescending protein is stressful for cancer cells. The advantage of a transfection with fluorescending proteins is the fact that cloned cells can be used for several generations and that there is no change in the biological behaviour of the cancer cells (4). Up to now, there are no reports on the usage of fluorescending HB cells in animals. The reason therefore might be that the growth behaviour of transfected tumor cells is inferior to that in untransfected tumor cells and that the establishment of HB xenografts is already difficult in non transfected cells (4,16). Further efforts will be required to establish stable xenografts of transfected human HB in animal models.

### 3.2. Optical imaging – Photodynamic diagnosis

Besides fluorescending proteins, photodynamic agents are another possibility for optical imaging in HB. Till et al. described a photodynamic videoscopic fluorescence diagnosis of peritoneal and thoracic metastases of HB in nude rats (17). Therefore, they injected human HB cells into the abdomen and thoracic cavity in these animals. After tumor growth of 7 weeks, a peritoneal lavage with 5-aminolevulinic acid (ALA) was carried out. 5-ALA is metabolized into protoporphyrin IX. Under excitation with blue light (380-450 nm), protoporphyn IX emits a red fluorescence at 640 nm. Afterwards, they were able to detect all lesions, which were seen with white light during videoscopy, also due to their fluorescending behaviour using a photodynamic diagnosis system. These findings were confirmed by a specific fluorescence peak at 635 nm measured by spectrometry. The authors concluded that HB photodynamic fluorescence diagnosis is feasible using 5-ALA (17,18).

The main problem of 5-ALA is that it is rapidly bleached out and exposure to light is limited to short illumination times (19). Therefore, other photosensitizers have been investigated. Hypericin, a hydroxylated phenanthroperylequinone derivate found in plants of St. Johns Wort, is another non- or weakly toxic photosensitizer (20). Hypericin induced fluorescence is stable and the fluorescence clearence is lower than in 5-ALA (19). Besides its use as a photodynamic agent for photodynamic therapy (PDT), the substance has also been used for in vivo detection of cancer cells. Most promising clinical effects have been described for the diagnosis of bladder carcinoma using cystoscopy (21). Hypericin was also used for an ex vivo fluorescence detection of bladder cancer by urine

cytology (22). Our group has demonstrated a hypericin uptake in epithelial liver tumor cells (HB and HCC) leading to a strong fluorescence signal (20). The role of in vivo tumor cell visualization through hypericin has not been analyzed so far.

### 3.3. Positron emission tomography (PET)

Other molecular imaging modalities have not been commonly studied in HB. F(18)-fluoro-deoxy-glucose positron emission tomography (FDG-PET) was first described by Philip et al. in recurrent hepatoblastoma. The authors reported that they were able to identify tumor recurrences which were not detectable by other conventional imaging modalities such as CT or MRI (23). Mody et al. demonstrated that F18-FDG-PET is probably most useful to assess response to therapy in AFP negative cases and to detect metastatic disease. Positive FDG-PET findings might be confused with non metastatic inflammatory processes like granulomas (24). Although this imaging modality seems to be important for HB, the number of cases is low and studies including large patient cohorts must follow to validate this technique.

### 3.4. PET-CT

PET-CT is an imaging technique combining the functional information of PET with the imaging of anatomical structures from computed tomography (CT). It is regularly used in oncology. PET-CT has already been studied in different pediatric solid tumors such as neuroblastoma, osteosarcoma, squamous cell carcinoma, synovial sarcoma, germ cell tumor, desmoid tumor, melanoma, rhabdomyosarcoma, Hodgkin`s lymphoma, non-Hodgkin-lymphoma, and Ewing`s sarcoma in low case numbers (25). The authors of the related study found that PET-CT was not outmatched in the detection of solid primary tumors and metastases compared to CT scans alone, but the specificity of PET-CT for the characterization of pulmonary metastases with a diameter > 0.5 cm and lymph node metastases with a diameter < 1 cm was significantly higher than in CT alone (25). In hepatoblastoma, there is only a single case report on successful detection of a recurrent tumor by PET-CT, which was not detectable by conventional CT or MRI (26). Besides these positive effects of PET-CT, there are disadvantages such as the limited soft tissue contrast and the non simultaneous data acquisition causing imaging artifacts. Additionally, a significant radiation dose is required (27). Through this, the working eligibility of PET-CT in patients with HB might be reduced. Nevertheless, the role of PET-CT is currently subject to intensive investigation and further studies will most likely assess this diagnostic tool in children with solid tumors.

### 3.5. PET-MRI

Realizing the disadvantages of PET-CT, other imaging modalities such as PET-MRI come into focus. PET allows high sensitivity tracking of biomarkers, but does not sufficiently resolve the morphology. MRI has a lower specificity, but produces high soft-tissue contrasts and provides spectroscopic as well as functional information (28). PET-MRI combines the advantages of both techniques and allows high resolution imaging of functional processes. Judenhofer et al. reported on the development of a three-dimensional animal PET scanner, which was built into a 7-T MRI. They were able to show that functional and morphological PET-MRI data could be acquired from living mice (28). PET-MRI seems to be more beneficial than PET-CT especially in studies, in which a high soft tissue contrast is required or high radiation doses should be avoided as it is the case in children (29). Up to now, there are only a very low number of scanners available worldwide, and they are only used for research purposes. In the next decade, whole body PET-MRI scanners are going to be developed allowing the evaluation of this novel, provoking technique in pediatric liver tumors also.

## 4. PHOTODYNAMIC THERAPY

Besides novel diagnostic imaging modalities, new treatment options are required for children suffering from advanced HB. Photodynamic therapy (PDT) is used as an anticancer treatment for different tumor entities such as bladder carcinoma, rhabdomyosarcoma, cholangiocellular carcinoma and glioblastoma (20, 30-32). Activation of the photodynamic drug using light of the appropriate wavelength leads to the development of singlet oxygen radicals inducing cytotoxic effects on cancer cells (33). Different substances such as 5-ALA or hypericin are regularly used for PDT. Hypericin seems to be currently the most promising substance for PDT as it offers stable fluorescence as well as a good tissue penetration (19). Hypericin was found to be internalized into cancer cells by partitioning, pinocytosis and endocytosis and is mainly found in the endoplasmatic reticulum (34). Hypericin is preferentially taken up in tumor cells and effects of PDT are stronger in tumor cells than in normal tissue (19,20). The authors have demonstrated that hypericin induced PDT was achieved in hepatoblastoma (HUH6, HepT1) and hepatocellular (HepG2) cancer cells (20). There was a dose-dependent uptake of hypericin in these tumor cells, which is essential for successful PDT. Major changes in cell morphology leading to destruction of cancer cells were caused by hypericin-induced PDT. A major mechanism of hypericin induced PDT seems to be apoptosis mediated via the p38 (MAPK) and PI3K signaling pathways (35). A complete apoptosis was found after incubation of 50 minutes and hypericin concentrations of 7.5 to 12.5 µM. PDT could not completely abolish proliferative activity of the tumor cells (20). Therefore, higher doses would be required for hypericin-mediated PDT in vivo.

In hepatoblastoma, high hypericin concentrations (12.5 µM) and illumination times of 50 minutes were required for a nearly complete disappearance of the cancer cells. In hepatocellular carcinoma cell lines, lower doses and illumination times could be used resulting in the same results. This observation might possibly be explained with the different tumor biology of the tumor cell lines (20).

Bergmann et al. investigated the effectiveness of 5-ALA induced PDT in a human hepatoblastoma cell line (HUH6 (36)). The basis of these studies is the fact that 5-ALA, a natural precursor in the heme biosynthesis, leads to

a selective accumulation of photoactive protoporphyrin IX within different neoplasm. Stimulation by light leads to emission of fluorescence as well as development of singlet oxygen molecules making photodynamic therapy possible. The authors described a 3 to 4-fold higher fluorescence intensity than in controls assuming a better uptake of 5-ALA in cancer cells. They also described that a reduction of cancer cell viability was depending on the applied light dose (36). Additionally, they performed an animal model in immunoincompetent rnu/rnu nude rats. Hepatoblastoma cells were injected into the peritoneum and the abdominal wall under laparoscopic guidance. After 7 weeks, 5-ALA was injected into the peritoneal cavity and tumors were irradiated with green light. They only found mild fluorescence within normal liver tissue and increased fluorescence at the implanted tumors. Histological work up of all irradiated tumors showed phototoxic necrosis (36).

A possible clinical application for photodynamic therapy of childhood HB consists of the intraoperative application during tumor surgery. Residual microscopic tumor rests at the resection margins could be approached. This would especially be helpful in difficult anatomical situations or extended liver resections.

Besides all advantages, the disadvantages of PDT have to be considered. One major disadvantage is the fact that the phototoxic agent is not specifically accumulated in tumor cells. However, the uptake of these substances seems to be higher in fast dividing cells making PDT especially useful in conditions, in which the tumor is surrounded by non dividing tissue such as brain tissue. Accordingly, 5-ALA was licensed for photodynamic assisted resections of malignant gliomas (37). Bergmann et al. also observed higher 5-ALA fluorescence within hepatoblastoma xenografts compared to normal liver tissue (36). Normal liver tissue in children has a high metabolic activity suggesting a high uptake of photodynamic agents. Therefore, discrimination between normal liver tissue and tumor tissue would be difficult during photodynamic diagnosis and activation of the photodynamic agent by light irradiation would possibly also affect normal liver tissue. Consecutively, methods have to be established in order to achieve tumor cell specificity of photodynamic agents for use in HB.

Side effects of photodynamic therapy might be another problem. Photodynamic agents, especially hypericin, might cause a severe photodermatitis, called hypericism, which was mainly described in grazing animals after ingestion of St. John`s wort and UV light exposure (38). In humans, concentrations of hypericin seem to be to low for induction of hypericism, but should be kept in mind during therapy (39). The side effects of 5-ALA seem even to be lower (40).

Taken together, photodynamic therapy might represent a promising approach for epithelial pediatric liver tumors. It can be either used for photodynamic diagnosis or photodynamic therapy in vitro and in vivo. Many problems are unsolved up to now. Therefore, further efforts must be made in order establish this novel therapy in clinical settings.

## 5. PERSPECTIVE

Molecular imaging is an important new field offering perspectives towards novel diagnostic and treatment approaches in hepatoblastoma. Many unsolved questions of this tumor entity may be addressed including tumor-host-interaction, metastatic invasion, angiogenesis, as well as investigation of novel treatment approaches such as immunotherapy. Elements of molecular imaging like PET-CT or PET-MRI will possibly find their way into the routine diagnostic workup of HB.

Photodynamic diagnosis and therapy seems to be a promising diagnostic and therapeutic approach in HB. First prosperities have been made, but further efforts need to be done in order to bring these basic research investigations into clinical trials.

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