Translational Research and Surgical Strategies of Childhood Solid Tumors

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Surgery is one of the major elements of therapeutic regimens for malignant solid tumors in childhood besides chemotherapy and irradiation and it represents the mainstay of treatment for benign tumors. Time and extension of tumor resection usually has its fixed place in most pediatric oncologic protocols. However, it is not clear whether this represents the optimal approach in every individual patient due to the large biologic varieties of many of these diseases. The surgeon has to keep in mind that he does not operate on an inanimate object but rather on a living neoplasm in a complicated organism. Therefore, tumor biology should influence the surgical strategy in the individual patient. However, until now there has not been undertaken very much surgery-related biological research on childhood tumors. In this review two questions are asked and answers shown with some examples:

- How can results of translational research influence surgical strategies for distinct tumor entities or individual patients?
- How can surgery itself influence the biological behaviour of childhood tumors?

Influence of translational research on surgical strategies

Molecular prognostic markers and surgical strategies. In recent years a large number of molecular genetic alterations have been detected in childhood malignancies (1). For some of these it could be shown that they are valid prognostic markers and can be used to define patient risk groups for differentiated treatment approaches. This may also effect the surgical strategy in some cases. In neuroblastoma a large number of prognostic relevant genetic and molecular genetic alterations have been described. The best known and most powerful of these is the amplification of the MYCN oncogene in the tumor cells. Until now there have not been undertaken many investigations analysing whether the amplification of MYCN should lead to a special surgical approach. In a large retrospective study on the German Cooperative Neuroblastoma Trials NB79 – 90 with 2251 patients we found that radical surgery with resection of more than 90% of the tumor mass does not improve long-term survival of the majority of patients with a neuroblastoma of any stage, but that those with an INSS stage 3 or 4 tumor with MYCN amplification have a better outcome after a radical resection (2). Currently, these results are validated with the data of the recent German study NB97. But the relevance of other well-known prognostic markers in neuroblastoma such as alterations of chromosomes 1p and 11q, Trk A and B, and others is still unknown. In Wilms tumor loss of heterozygosity for chromosomes 1p and 16q have been shown to be adverse prognostic factors in favorable-histology tumors, especially when they occur combined (3). Should surgery for these tumors be more radical than in others and should it be done exclusively after neoadjuvant chemotherapy? Can nephron preserving surgery be promoted in favorable-histology Wilms tumors without these molecular genetic alterations? Until now there exist no valid data to answer these questions, nor investigations on other recently found aberrations such as overexpression of the CACNA1E transcript (4). For hepatoblastoma Cairo et al. (5) very recently found a 16-gene signature with a highly significant prognostic relevance and we identified further putative prognostic molecular markers, but the relevance of these findings for surgery is not known.

Molecular markers for differential diagnosis and surgical strategies. During the last years a large number of cytogenetic and molecular genetic alterations have been identified in malignant soft tissue sarcomas. These can
help in establishing the differential diagnosis between these tumors especially in cases with very undifferentiated and unclear histology and immunohistochemistry (6). This will then influence the chosen treatment regimen and should also be taken into account for planning of surgery. It can well make a difference for the surgical approach whether a highly malignant tumor with unclear histology is categorized as rhabdomyosarcoma, extraskeletal Ewing sarcoma or rhabdoid tumor, since it will determine timing of surgery, as well as the attempted radicality in relationship to the accepted risk for complications and mutilation. New markers will be found in these tumors and prospective studies are needed to determine the relevance of these for surgical strategies.

Invasive growth. Some aggressive malignant tumors such as renal tumors, sarcomas and hepatoblastoma display invasive growth into adjacent organs and blood vessels, which affects surgical strategy and can make the utilization of special surgical techniques necessary. Some neuroblastomas however, which undergo differentiation from small blue round cell histology to ganglioneuroblastoma during chemotherapy also invade blood vessel walls. In these cases differentiated neuroblastoma cells or ganglion cells can be detected between the layers of vessel walls. It is unknown, what the stimulus for this invasion or migration of the tumor cells can be, but this leads to the situation that during surgery the tumor tissue cannot be cleanly dissected from the blood vessels and the risk for their rupture is highly increased. Therefore, in such tumors a radical tumor resection is often not possible without major injury of important vessels (7). In one of our projects we try to find molecular markers with which these specific neuroblastomas could be identified from a biopsy at the time of diagnosis, making a better planning of the final surgery possible.

Anticancer drug resistance. Multiple drug resistance can be found in many malignant childhood tumors, either existing already at the outset of treatment or becoming apparent during the course of disease. Several molecular mechanisms can be responsible for drug resistance, mainly the inhibition of drug accumulation in the tumor cells, drug detoxification or altered affinity of the intracellular targets, DNA repair mechanisms and the inhibition of apoptosis (8). The knowledge of drug resistance in a tumor will primarily influence the choice of cytotoxic drugs and maybe the application of chemosensitizers, but it should also be taken into account for planning surgery. Here, resistant tumors may need earlier and more aggressive surgery in comparison to tumors which show a steady response to chemotherapy over a long period. For hepatoblastoma we found that the prognosis is reduced in patients in whom the tumor resection is performed after development of multiple drug resistance (9). Therefore, routine analyses of molecular resistant mechanisms in malignant tumors may enable a better tailoring of surgery in the future.

The influence of surgery on tumor biology

Healing from surgical injury and tumor growth. Surgical operations are always associated with injury of the skin and deeper structures. This implicates the process of wound healing, which develops in several phases: hemostasis, inflammation, proliferation and remodeling. These phases are strictly regulated. Of special interest here is the proliferative phase, which is characterized by angiogenesis, collagen deposition, formation of fibrous tissue and epithelialization. All these steps are made possible by proliferation of specified cells, which again is inaugurated and controlled by growth factors. The most important of these are EGF, TGF-alpha, HGF-SF, VEGF, PDGF, FGF-1 and - 2, TGF-beta and KGF. There exists ample research activity on the role of these growth factors and their receptors in healing processes and they are also well known to have a role in many benign and malignant tumors. Also it is well acknowledged that there are many biological similarities between healing processes and tumor growth (10). Thus, it seems obvious that surgery and the consecutive induction of the healing process can also affect the behavior of eventual residual tumor through the involved growth factors. This can well be an activation of tumor cell proliferation and migration, suppression of apoptosis and angiogenesis. Although it is well known that many malignant and benign tumors show an expression or even an overexpression of the receptors for the above.
mentioned growth factors, there exist almost no knowledge on this interaction after tumor surgery. From clinical observation of several cases we know that benign neurofibromas in children with neurofibromatosis type I (NF I) can react with rapid growth during the first weeks after incomplete resection to at least the former extension. It has been found that neurofibromas express receptors for several of the growth factors involved in healing, especially those for EGF, VEGF and possibly also PDGF (11). However, the role of these receptors and their ligand for post-surgical growth is still unclear. The same accounts for the question whether incomplete surgery of neurofibromas in NF I patients can maybe even enhance the transformation to malignant peripheral nerve sheet tumors. An early medication with an antiproliferative agent such as rapamycin might be a clinical solution in this situation, but there exist no valid studies on this approach.

Furthermore, fractures as well as surgical trauma of the bones lead to a similar healing process during which the activation of osteoblasts and fibroblasts are important for the formation of new bone. This process is also induced under the influence of growth factors, mainly bone morphogenic proteins (BMP), FGFs, PDGF and TGF-beta. Research activities during the last years revealed that these growth factors also have an influence on proliferation and invasion of osteosarcoma cells (12, 13). This may be an explanation for the observation of the development of distant metastases after resection of osteosarcomas, especially in former times, when preoperative chemotherapy was not routinely used in this tumor.

**Enhancing tumor cell migration by surgery.**

Minimal invasive surgery is increasingly utilised for taking biopsies and resection of tumors both in the thorax and the abdomen. This technique is very attractive, since surgical trauma is reduced and recovery faster in comparison to open surgery. However, the biological behaviour of neoplasms may be influenced by setting a pneumoperitoneum or pneumothorax with an artificial high pressure. Recently, it was shown that CO2 pneumoperitoneum increases systemic tumor spread in murine neuroblastoma by facilitating tumor cell migration (14). Very little is known about such interactions but the first preliminary results indicate that caution is indicated when using new surgical techniques in malignant childhood tumors.

**Organ regeneration and tumor growth.**

Besides inducing wound healing, partial resection of solid organs can also result in complete or partial regeneration of the organ especially in young children. Thus, surgery induces a process of initial cell proliferation and consecutive tissue organisation, which is again controlled mainly by differentiated expression of growth factors and their receptors. The most prominent example for solid organ regeneration is that of the liver after partial hepectomy, which in young children starts only some days after surgery and is terminated after 6 – 8 weeks. This process has been intensively investigated during the last 20 years and it is now clear that two mitogenic signals are mainly involved in induction and maintaining hepatocyte proliferation: the hepatocyte growth factor – scatter factor (HGF-SF) with the MET receptor and the epidermal growth factor (EGF) with the EGF-receptor (EGFR) together with the other less prominent EGFR ligands TGF-alpha, heparin binding-EGF and amphiregulin (15). Since in a number of young children with extended hepatoblastoma we had to observe rapid growth of lung metastases and sometimes also of local recurrent tumor after liver surgery, we asked whether the induction of liver regeneration can in parallel also induce tumor cell proliferation. Hepatoblastoma cells, which have many common characteristics with fetal or embryonal liver cells, also show a strong expression of MET and EGFR. In a series of investigations we found a highly increased secretion of HGF-SF in children during the first days after major abdominal surgery and especially after hepatic resections. It also became clear that HGF-SF in vitro leads to a dose-dependent increase of viable tumor cells (16). Further research demonstrated that HGF-SF is a strong mediator of tumor cell scattering and migration (17) and inhibits apoptosis through different intracellular signalling pathways, but alone does not directly enhance proliferation of the tumor cells (18). Since also in hepatocytes both HGF-SF and EGF seem to be necessary for sustained proliferation during liver regeneration, our further research will
concentrate on the combined effect of these and other growth factors on growth of hepatoblastoma and possible approaches to inhibit such interactions. Besides the fact that the phenomenon of surgery stimulated growth of a malignant tumor by induction of regeneration of the tumor's original organ is biologically interesting, this observation is important for planning surgery. We therefore advise that liver resection for extended hepatoblastoma should only be performed after administration of effective chemotherapy. Furthermore, our investigations will show whether other agents targeting important molecular pathways are effective in stopping hepatoblastoma growth and can still be applied peri-operatively to the patients. These can be drugs, which we found to inhibit hepatoblastoma cell proliferation in vitro by blocking the IGF-Akt-mTOR- such as rapamycin (19), the hedgehog-pathway (20), or the WNT-pathway (i.e. non-steroidal anti-inflammatory drugs, NSAID; 21).

**Conclusion**

Some results of molecular research in tumors of childhood have an impact for the development of better surgical strategies and techniques and therefore translational research should also focus on the surgical relevance of biological findings. Also, surgery itself can influence the biological behavior of some childhood neoplasms and these interactions should become a focus of research activities in pediatric surgical oncology.

**References**