RESEARCH CONFERENCES

ESF-EMBO Symposium

Molecular Biology and Innovative Therapies in Sarcomas of Childhood and Adolescence

29 September - 4 October 2012

Polonia Castle in Pultusk, Poland

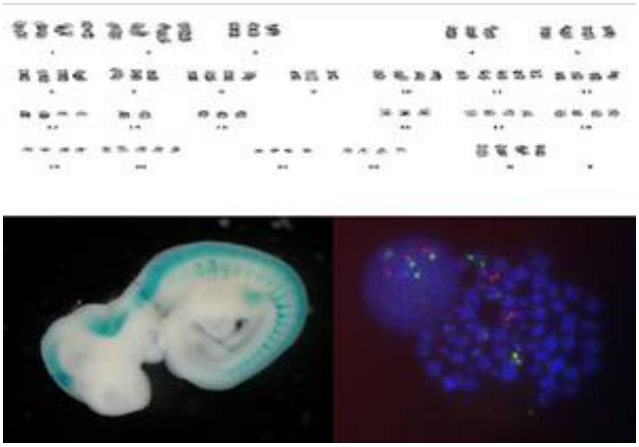
Chaired by: Professor Beat Schäfer, University Children's Hospital, CH

Co-Chaired by: Professor Ewa Koscielniak, Olgahospital, DE

Scientific organising committee: Prof. Simone Fulda, Institute for Experimental Tumour Research in Pediatrics, DE; Prof. Heinrich Kovar, Children's Cancer Research Institute, AT

http://www.esf.org/conferences/12393

Highlights & Scientific Report



Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

Pediatric sarcomas are rare tumors with a dismal prognosis when presenting at diagnosis with metastasis. To advance research and therapy of these tumors, this conference brought together leading biologists and clinicians involved in early clinical trials to implement an interdisciplinary approach to manage these diseases.

Traditionally, bone-related sarcomas such as osteosarcoma and Ewing sarcoma held separate research conferences from the ones dealing with soft tissue sarcomas that are also not focused on pediatric patients only. This conference was the first to specifically concentrate on two groups of sarcomas, namely rhabdomyosarcoma and Ewing sarcoma, in the pediatric and young adult population. The rational is given by the common genetic background of these diseases which are characterized by chromosomal translocations generating fused transcription factors with aberrant transcriptional activity. This allowed gathering most leading principal investigators active in this field which reflected the high scientific standard of all oral and poster presentations.

Since the chimaeric transcription factors are often the only genetic abnormality present at high frequency in these tumors, they are hypothesized to be one of the tumor initiating events. Therefore, a large effort is still directed at identification of specific, either activated or repressed target genes. Several speakers discussed novel techniques such as next-generation sequencing and whole genome ChIP-seq to identify such target genes including mechanisms of transcriptional regulation. Several new targets genes that have now been individually characterized were presented an discussed as novel therapeutic targets at the conference.

Another important area in this field is the development of genetic animal models to further advance our understanding of the biology of the diseases and to pre-clinically evaluate novel targeted compounds. A specific session addressed the progress in this field spanning from mouse to zebrafish models. Especially for Ewing sarcoma, the lack of an appropriate mouse model still represents a major bottleneck.

Two discussion sessions identified future areas of importance. These included better incorporation of developmental biology leaders to make progress in identification of the cell of origin and comparison with normal differentiation processes. The importance of (deregulated) embryonal signaling pathways for the tumorigenic process of pediatric sarcomas is increasingly recognized. Additional bottlenecks identified were availability of biomaterial, a possible common internet platform for data sharing and exchange, and criteria to select novel therapeutic targets including identification of synthetic lethal interactions.

Finally, the conference was judged by all participants to be a big success and everybody unanimously agreed to re-apply for a second conference under this format in 2014.

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Scientific Report

Executive Summary

(2 pages max)

What is the rationale to organize a common conference for the two pediatric sarcomas RMS and Ewing sarcoma? Both are rare tumors of possibly mesodermal origin that represent only few % of cancers in the pediatric population. Although sarcomas are a very heterogenous group of tumors, they have one common genetic background which is the presence of a specific chromosomal translocation in the majority of the cases. These translocations generate a fusion protein which is composed of two transcription factors (TF). Typically, one TF is a developmentally regulated factor that is essential for proper specification of a given lineage and provides the DNA binding domain while the partner TF contributes a transactivation domain. Therefore, the genetic basis of these diseases is thought to be transcriptional deregulation of normal developmental processes. It is also generally accepted that development of novel treatment approaches will require a more detailed understanding of the biology of these oncogenic TF and the underlying biology of tumor cells. Importantly however, available mouse models using PAX3/FOXO1 as oncogenic driver and attempts to generate a mouse model based on expression of EWS/FLI1 unravels the importance of largely unknown secondary hits which are required for tumorigenesis. Identification of such secondary hits likely requires large scale next generation sequencing of which several projects are now getting under way. One remaining challenge in this area is the availability of sufficient and high quality patient material.

Since transcription factors are thought to be undruggable based on their structure, novel treatment approaches likely have to be indirect. One such indirect possibility might lie in the epigenetic control of gene expression which seems to play a major role also in sarcomas. Additional areas that are beginning to emerge are the detailed characterization of the role that is played by embryonal signaling pathways such as wnt, notch and sonic hedgehog in tumorigenesis of sarcomas.

One additional and important highlight of the conference were the poster sessions. A total of 35 posters were displayed on two different evenings. Poster sessions were scheduled to last for two hours, however quite some people were still discussing after this, allowing also students to establish new connections with PIs and discuss future collaborations. These become increasingly important to achieve substantial progress in a research field with limited resources.

Scientific Content of the Conference

(1 page min.)

- *Summary of the conference sessions focusing on the scientific highlights*

- *Assessment of the results and their potential impact on future research or applications*

The first session of the conference was to clinical research. Uta Dirksen gave an overview on current treatment of Ewing sarcomas in Europe. Similarly, treatment of RMS was discussed by Ewa Koscielniak. Some discrepancy in the field concerns the clinical outcome of different PAX/FOXO1 fusions. While different outcomes have been found in the US, the European CWS trials does not see this (S. Stegmaier). Finally, a novel fusion transcription factor, EWS/NFAT2c was describe in Ewing (K. Szuhai).

The conference then moved to some basic biological aspects. Different levels of gene regulation were discussed by H. Kovar who also investigated the role of the notch pathway in Ewing sarcoma. O. Delattre described their attempts to generate a mouse model for Ewing sarcoma and also identified an additional novel translocation, BCOR-CCNB3. The next two talks looked at specific aspects in RMS, namely the biological role of CB1, a well known downstream target gene. Interestingly, inhibition of CB1 abrogates lung metastasis in a xenograft mouse model (G. Grosveld). Finally, S. Hettmer reported on a promising in vitro system that allows to transform primary satellite cells by specific oncogenes such as ras together with deletion of CDKN2A.

Transcriptional control and epigenetics was the theme of the next session. S. Lessnik discussed a novel target gene repressed by EWS/FLI1, namely lysyl oxidase. For transcriptional repression, association of EWS/FLI1 with HDACs and the NuRD complex was found to be necessary. As a compontent of the wnt signaling pathway, DKK2 was described to be highly overexpressed in Ewing sarcoma. DKK2 expression is critical for malignant cell outgrowth in vitro and in vivo (G. Richter). Apart form histone acetylation, also histone methylation is an important epigenetic mechanism contributing to genetic heterogeneity. E. Lawlor described studies in which she investigated the role of the polycomb repressor complex in response to EWS/FLI1 expression. Finally, activity of the fusion transcription factors might also be regulated by protein-protein interactions. One interesting co-factor to follow up in the future might be E2F (R. Schwentner).

An important tool to further understanding tumor biology but also to prioritize drugs for pre-clinical development is the availability of genetically engineered animal models. While no mouse model is available up to now for EWS/FLI1, C. Keller described mice expressing PAX3/FOXO1 that will form aRMS tumors in collaboration with p53 knock-out. Interestingly, he used different driver genes along the myogenic lineage to initiate expression of the fusion protein and found the most penetrating model to be with myf6. From this data, he concluded that the cell of origin for aRMS is a fetal myoblast. In contrast to aRMS, several mouse models are available that give rise to eRMS, the RMS subtype without expression of the fusion protein. One the important one involves activation of the shh pathway (H. Hahn). An interesting zebrafish model utilizing activated k-ras was discussed by D. Langenau. This model allows in vivo imaging of developing tumors. While not all cells in a tumor might have tumor-propagating potential, these cells clearly can enter the vasculature and play a critical role in metastasis, at least in zebrafish.

In the next session, less well studied and for the most part novel pathways in sarcoma biology were discussed by several speakers. T. Triche discussed the prognostic significance of non-coding RNA identified on the human exon array. In Ewing sarcoma, Erbb4 might play a role in metastasis (P. Sorensen) while cul4, an E3-ubiquitin ligase, might have a general role in ES (C. Mackintosh). Another novel tumor suppressor in ES might be lysyl oxidase (J. Alonso). In RMS, novel pathways that were discussed included the role of pro-protein convertases that are involved in processing of important therapeutic targets such as IGF1R (M. Bernasconi). Several novel target genes of PAX3/FOXO1 were characterized by different groups such as carnitine palmitoyltransferase and pleiotrophin (T. Chen), JARID2 (J. Shipley) and p-cadherin (C. Gauthier-Rouviere).

The theme of the conference then moved from targets of the fusion gene to the fusion gene itself. J. Toretsky discussed interaction of EWS/FLI1 with RNA helicase A which can be utilized as therapeutic target and the improvements that were made in developing a specific small therapeutic molecule. Another approach was described by B. Schäfer who investigated post-translational modifications of PAX3/FOXO1. Several phosphorylation sited were identified and a candidate upstream kinase (PLK1) that could regulate PAX3/FOXO1 activity was identified. The same phosphorylation sites were also investigated biochemically in much more detail by A. Hollenbach. Finally, the role of gene amplification in the pathogenesis of RMS was addressed by F. Barr.

Several important signaling pathways were highlighted in the next session. These included a CRKL/Yes axis (L. Helman), targeting of c-met by a small molecule inhibitor (S.J. Ding) and the influence of the notch pathway on invasion of RMS cells (J. Roma). The possibility of targeting both tumor and stromal cells by inhibiting the PDGFR pathway was brought up by M. Ehman. A new exciting area of research are the newly discovered ALK mutations that occur in RMS and which can be targeted by small molecule inhibitors (Y. Versleijen-Jonkers). An important new area for sarcoma biology is the role of miRNAs in the tumorigenic process. Some miRNA termed the myomiRs are important for myogenic differentiation and hence RMS biology. These include miR206 and miR1 which were discussed by S. Subramanian and C. Ponzetto.

The last day of the conference was devoted to different resistance mechanisms and cancer stem cells. Unfortunately, characterization of cancer stem cells in sarcoma is still in its infancy. Attempts to define a subpopulation by side-population analysis in Ewing sarcoma were discussed by M. Hotfilder. Important for resistance to given treatments are also mechanisms of apoptosis. Indeed, sensitivity to treatment with TRAIL was reported by two researchers (S. Fulda and F. Redini). Along similar lines, blockade of surviving might enhance sensitivity to therapeutic T cells (K. Simon-Keller). Genome wide ChIP-seq. experiments could also help to identify important downstream targets that mediate resistance. Such an experiments was presented by L. Cao for PAX3/FOXO1. Among the important receptor targets that he identified were FGFR4, c-met, IGFR1R and DR5. Interstingly, about 25% of the identified binding sites seem to lie in the first intron and some even further downstream within genes. P. Zammit described an in vitro culture system of isolated single muscle fibers that contained satellite cells in their natural niche. Ectopic expression of PAX3/FOXO1 was able to induce expression of myogenin but not myoD. However, it remained unclear whether the satellite become fully transformed. D. Loeb then looked at another interesting gene in the context of Ewing sarcoma, namely WT1. This transcription factor might play an important role for angiogenesis in this tumor.

The last session of the conference then closed the circle to discuss potentially novel treatment options. First in this session was P.Houghton who summarized the effort made by the PPTP program to identify important novel drugs for sarcoma treatment. Interestingly, response rates for known drugs in his xenograft models is 30% while it was only 9% for experimental drugs. For sarcomas one important class of drugs might be topoisomerase I inhibitors. Disappointingly, inhibition of the IGF1R/PI3K axis did not result in significant reduction in growth rates. He concluded that in vitro data does not necessarily predict in vivo response and kinase inhibitors in general have only modest activity. Since the PPTP program works with animal models, it will be crucial to develop strategies for clinical translation. In sarcoma treatment, there are at the moment no targeted agents in use. J. Chisholm described the structure that is used in Europe to achieve this goal, which is called ITCC (innovative treatments for children with cancer). Several trials are now implemented under this umbrella. One of the targets that is currently being developed

at the Royal Marsden was discussed in the final talk of the meeting by S. Gatz, namely FGFR which was found mutated in some aRMS and overexpressed in most of them.

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

The meeting included two forward look sessions. They were held in the format of plenary discussions where all participants were able to provide their input. Both sessions lasted for 1,5 hours, were attended by all participants and very lively. Topics to be discussed were identified beforehand and written on the board. Both sessions were anchored by H. Kovar. The first important topic was the authentity of the cell lines used by the community. Since several names have been given in the past to the same cell lines, not everybody uses the lines properly. The summary of the discussion was the following cell line nomenclature, whereby cell lines within semi-colons are identical: Rh3 - Rh28; Rh30 - RMS13; RD - TE671 and all clones called RD; Rh4 - Rh41; BIRCH - Rh36; two Rh30 cells exist (one with and one without fusion); two Rh18 cells exist (one with and one without fusion); A673 is a Ewing sarcoma cell (not RMS); Rh1 - EW8 (are also Ewing cells); A204 is a rhabdoid cell (and not a RMS); CT10 – SMSCTR.
These clarifications will be very helpful to the field in the future and should also be included in a review that is being prepared for publication by Ch. Keller. Next, several research topics were addressed. These included identification of genetic alterations, role of the micro- and macroenvironment, significance of tumor heterogeneity and pharmacogenomics. The community agreed that novel approaches are needed to improve our techniques currently in use like cell tracking, new animal models and new culture models that depend less on plastic dishes. Since cell of origin for these sarcomas is still unclear, approaches used by developmental biologists might be interesting as well. Lee Helman announced the organization of the first AACR special conference on pediatric cancer in November 2013 in San Diego.
In the second discussion round, the topics included more translational approaches. How much pre-clinical data is needed to justify a phase I trial? What is a good druggable target? It was a consensus opinion that going after individual targets might be old-fashioned. However, it was also pointed out that in contrast to adult carcinomas, the mutation rate in pediatric sarcomas is quite low. Hence, there are not that many mutations that you can identify and subsequently target with novel drugs. Therefore, we might put more effort into understanding the biology of the fusion proteins which are the only obvious mutations present in the sarcomas. In addition, we do not know much about the tumor evolution from primary tumor towards metastasis. This is mainly due to lack of appropriate patient material. Although this is recognized as an important bottleneck in research, there is really not much we can do to change it. Since we deal with a small patient population, the number of research groups active in the field is also limited and there are numerous obvious questions that need to be answered in the future. One of the emerging topics is therefore also to attract young research to this field. Organization of such workshops focussing on the biology and research aspects is one of the initiatives that we agreed to keep up in the next years.

- Is there a need for a foresight-type initiative?

Participants recognized a need to establish an electronic platform to facilitate exchange of data between the different laboratories and availability of genomic data to everybody. As possiblities, we can see development of a specific website devoted to pediatric sarcomas, but also discussion groups on social media such as linkedIn might be worth looking into.

Business Meeting Outcomes

- *Election of the Organising Committee of the next conference*
- *Identified Topics*
- *Next Steps*

The plenum decided that it would be highly desired to held a next conference under the same scheme in two years time. Therefore, we elected a next chair- and co-chairperson which were Heinrich Kovar from Vienna acting as chair, and Olivier Delattre from Paris acting as co-chair. An application for a conference to be held in 2014 was therefore submitted by the deadline to the ESF office.
The main topic of the conference will again be pediatric sarcomas. For the next conference, inclusion of osteosarcoma is planned. In addition, it was felt that a few high-level talks covering developmental biology would be stimulating, since sarcoma development has a clear interface with developmental processes.

Atmosphere and Infrastructure

- *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

Participants were very happy not only about the scientific part of the conference but also about the friendly atmosphere of the location, the quality of the rooms and the quality of the food (with few exceptions only). I received several mails and letters after the conference from participants thanking specifically for the organization of the first event ever focusing on pediatric sarcoma. Hence, I also would like to thank the ESF-EMBO for their support of this topic and to the conference officer who did a great job in giving a hand to people's questions and organizing additional individual requests.

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Date & Author:

February 8, 2013 Beat W. Schäfer, PhD