News Release

Title

A Gradual Transition Toward Anaplasia in Wilms Tumor Through Tolerance to Genetic Damage

Key Points

•Anaplastic Wilms tumor cells have a high ability of proliferation despite a high burden of genetic damage.

• Gradual transition toward anaplasia in Wilms tumor through accumulation of genetic aberrations.

• *TP53* loss of heterozygosity (LOH) can contribute to tumorigenesis even in the presence of a wild-type allele.

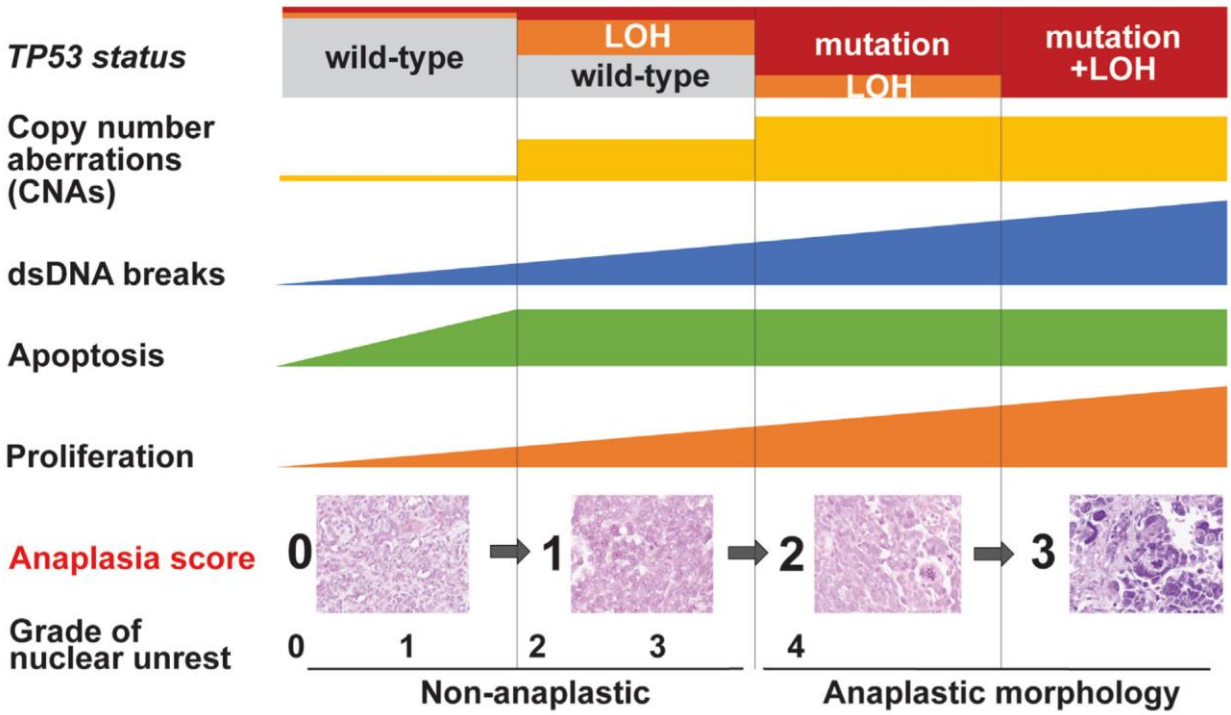
Summary

Patients with Wilms tumor (WT) in general have excellent survival, but the prognosis of patients belonging to the subgroup of WT with diffuse anaplasia (DA) is poor due to frequent resistance to chemotherapy. We hypothesized that DA WT cells might undergo changes, such as acquiring a persistent tolerance to DNA damage and copy number aberrations (CNAs), which could eventually lead to their resistance to chemotherapy treatment.

Tissue sections from chemotherapy-treated DA WTs (n=12) were compared with chemotherapy-treated nonanaplastic WTs (n=15) in a tissue microarray system, enabling analysis of 769 tumor regions. All regions were scored for anaplastic features and immunohistochemistry was used to quantify p53 expression, proliferation index (Ki67), and DNA double-strand breaks (rH2AX). CNAs were assessed by array-based genotyping and *TP53* mutations using targeted sequencing.

Proliferation index and the frequency of DNA double-strand breaks (rH2AX dot expression) increased with higher anaplasia scores. Almost all (95.6%) areas with full-scale anaplasia had *TP53* mutations or loss of heterozygosity, along with an increased amount of CNAs. Interestingly, areas with wild-type *TP53* with loss of heterozygosity and only one feature of anaplasia (anaplasia score 1) also had significantly higher proliferation indices, more DNA double-strand breaks, and more CNAs than regions without any anaplastic features (score 0); such areas may be preanaplastic cell populations under selective pressure for *TP53* mutations.

In conclusion, we suggest that chemoresistance of DA WTs may be partly explained by a high proliferative capability of anaplastic cells, which also have a high burden of double-stranded DNA breaks and CNAs, and that there is a gradual emergence of anaplasia in WT.



Research Background

Wilms tumor (WT), also known as nephroblastoma, is the most common childhood renal neoplasm. The survival rate of WT patients has been increasing over the past decades due to standardized multimodal treatment protocols, and the overall survival rate has recently reached 90%. However, 5–10% of WT patients are diagnosed with diffuse anaplasia (DA). The prognosis for patients with DA is unfavorable and the recurrence rate is high. This is likely due to the fact that the cancer cells are resistant to chemotherapy. It is well known that *TP53* mutations seem to play a key role in developing DA. On the other hand, the rates of *TP53* mutation in DA WT are very different among studies – ranging from 48% to 86%. One reason for these differences, is limited sampling of tumors and that the genetic analyses were performed without detailed pathological evaluation at the sampled areas. The biology underlying treatment-resistance in DA and the pathogenesis behind the emergence of DA in WT, remain insufficiently explored. To understand when and how DA WT appears could be a further improvement for the children with WT.

Research Results

Most tumor regions are non-anaplastic in DA WTs, explaining discrepant results in previous studies

In this study, we included 12 cases of DA WT, 5 cases of blastemal type WT, and 10 cases of intermediate risk WT. Among of these tumors, we collected a total 787 samples for anaplasia scoring to precisely map the anaplastic area in each tumor using a TMA system. Only 8.4% of areas (TMA cores) were scored as anaplastic (anaplasia score 2 or 3). Even in cases of DA WT, only 26.2% of areas showed anaplastic histology. Thus, in DA WT, most of the tumor histology is non-anaplastic and multiregional sampling seems to be necessary when selecting tumor tissues for a representative genetical analysis. The rates of *TP53* mutation in DA WT were reported from 48% to 86% in previous studies. Our results indicated that these differences in *TP53* mutation rate among studies could derive from sampling errors in DA WT. To systematically detect anaplastic histology for further genetic studies, anaplasia scoring could be a useful tool.

Anaplastic WT cells have a high ability of proliferation despite a high burden of genetic damage

It is well known that DA WTs show a relative resistance to chemotherapy and high recurrence rate. But the reasons for this have not been uncovered. In this study, we assessed proliferation and double stranded (ds)DNA damage through IHC detection of the Ki67 and γH2AX proteins combined with anaplasia scoring in DA WT. As the anaplasia score increased, the Ki67 proliferation index also significantly increased, implying that anaplastic cells had high ability of proliferation despite having undergone recent chemotherapy. On the other hand, the dot staining of γH2AX, which represents focal dsDNA damage, was also significantly increased as the anaplasia score increased. Moreover, the number of CNAs significantly increased as anaplasia score increased. These results indicate that chemoresistance of DA WTs may be partly explained by a high proliferation capability of anaplastic cells even though they also have a high burden of dsDNA breaks and CNAs.

Gradual transition toward anaplasia in WT through accumulation of genetic aberrations

We tried to reveal when and where anaplastic histology appeared in WT through detailed examination using anaplasia scoring. It is well known that nuclear accumulation of p53 proteins is correlated with the certain *TP53* point mutations. In this study, p53 expression was significantly increased in anaplastic cells (anaplasia score 2 or 3). When analyzing *TP53* mutation by

targeted deep sequencing, all areas showing complete anaplastic histology (score 3) had *TP53* mutation, while almost all areas showing non-anaplastic histology (score 0 or 1) did not have a mutation. These results indicate that *TP53* mutations could have a role in allowing, even driving, and high proliferative ability despite a high burden of dsDNA damage and CNAs. On the other hand, compared to areas with score 0, areas with score 1 had significantly higher proliferation and also a significantly higher burden of dsDNA breaks and CNAs while most of these areas were *TP53* wild type. However, areas with score 1 (hyperchromasia only) had significantly higher *TP53* loss of heterozygosity (LOH) compared to score 0 areas. We concluded that cells with morphology in the greyzone between non-anaplasia and anaplasia, also show molecular features of indicating they are in a transitory state towards anaplasia. In these cells, a low but significant degree of DNA damage could create a selection pressure for *TP53* mutation. After acquiring *TP53* mutation, such cells can then proliferate at an even higher rate, despite contracting a high amount of dsDNA damage and CNAs, leading to full-scale anaplastic histology. Our results indicate that WTs “grow bad” (develop anaplastic histology) rather than are born bad as previously indicated by other studies from our research group.

Research Summary and Future Perspective

This Study suggested that anaplastic histology gradually emerges from non-anaplastic areas by accumulating burdens of dsDNA damage and CNAs, often including *TP53* LOH. Such conditions can create selection pressures for *TP53* mutation. When WT cells acquire *TP53* mutation, they can grow under chemotherapy, even with a very high burden of dsDNA damage and CNAs, which correlate with chemotherapy resistance and typical anaplastic histology. However, we did not include focal anaplasia owing to the lack of available material. We believe that the difference between focal and disuse anaplasia is simply the relative area and distribution of anaplasia, rather than a difference in cancer cell biology. Further studies are necessary to confirm whether our results are applicable to focal anaplasia. We also need to consider the treatment strategies for DA WT. Most chemotherapies induce DNA damage (including dsDNA breaks) and cause tumor cells apoptosis. However, the cells with anaplastic histology could continue to grow with a high level of DNA damage. These characteristics can be associated with *TP53* mutation and the accumulation of mutated p53 proteins. Our subsequent investigations will reveal the molecular mechanisms that allow growth with a high burden of DNA damage in further detail. Revealing these mechanisms could be a breakthrough to overcome resistance to chemotherapy.

Publication

Journal：Modern Pathology
Title：A Gradual Transition Toward Anaplasia in Wilms Tumor Through Tolerance to Genetic Damage

Authors and affiliations

Kaname Uno[a,b], Bahar Rastegar[a], Caroline Jansson[a], Geoffroy Durand[a], Anders Valind[a,c], Subhayan Chattopadhyay[a], Alessia Bertolotti[d], Sara Ciceri[e,f], Filippo Spreafico[g], Paola Collini[h], Daniela Perotti[e,f], Linda Holmquist Mengelbier[a], David Gisselsson[a,i,j]

a. Division of Clinical Genetics, Department of Laboratory Medicine, Lund University, Lund, Sweden
b. Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Nagoya, Japan
c. Childhood Cancer Center, Skåne University Hospital, Lund, Sweden
d. Diagnostic and Molecular Research Lab, Department of Advanced Diagnostics, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
e. Molecular Bases of Genetic Risk and Genetic Testing Unit, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
f. Predictive Medicine: Molecular Bases of Genetic Risk, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
g. Pediatric Oncology Unit, Department of Medical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
h. Soft Tissue Tumor Pathology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
i. Division of Oncology-Pathology, Department of Clinical Science, Lund University, Lund, Sweden
j. Division of Clinical Genetics and Pathology, Department of Laboratory Medicine, Lund University Hospital, Skåne Healthcare Region, Lund, Sweden

DOI: 10.1016/j.modpat.2023.100382

Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Mod_231228.pdf