The PIDDosome in promoting p53-induced tumor suppression

Introduction

In diploid organisms, including humans, somatic cells can adopt polyploid states by whole genome duplication that are critical for organ development and function while providing a better chance to adapt to environmental changes. A potential challenge limiting adaptability arises however from problems in faithful chromosome segregation in proliferating polyploidy cells. Moreover, polyploidization is often associated with the development of aneuploidy leading to human disease, most prominently cancer. Of note, tetraploidy, micronucleation and chromosomal instability are frequently observed to precede genomic instability and aneuploidy early in tumor development. Common to normal as well as pathological polyploidization events is an accompanying increase in centrosome number which challenges dividing cells because they can foster aneuploidy during multipolar cell division or compensatory centrosome clustering. Typically, cells with extra centrosomes withdraw from the cell cycle or undergo apoptosis. However, until recently it remained unclear how this regulated. We have recently shown that the PIDDosome is necessary and sufficient to induce p53-stabilization and subsequent cell cycle arrest in response to supernumerary centrosomes. With this project we aimed to answer whether the PIDDosome acts as a first barrier towards centrosome-amplification related transformation pressure.

Results

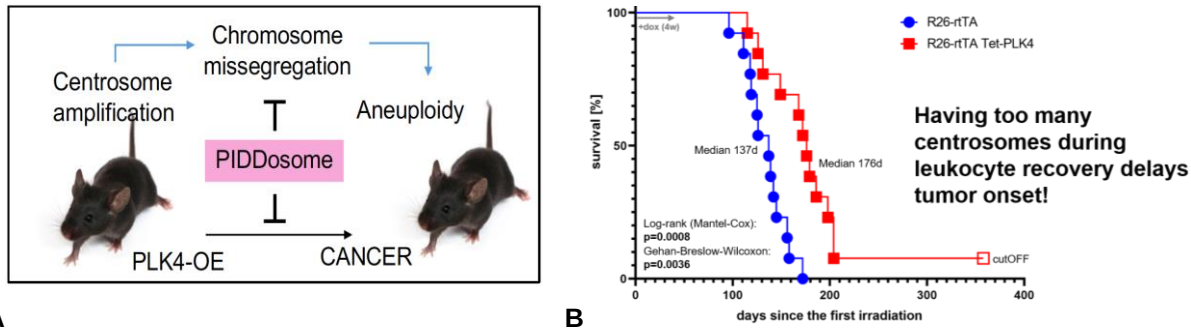

Fig. 1 Extra centrosome induced cancerogenesis A) Scheme on how the PIDDosome is thought to halt malignant transformation. B) *R26-rtTA Tet-PLK4* mice (red) have a delayed tumor onset as compared to littermate control *R26-rtTA* mice (blue).

To induce centrosome-overduplication we took advantage of TET-responsive, doxycycline-inducible PLK4-EYFP transgenic mice (kindly provided by the Holland lab). Continuous doxycycline administration in these mice was already shown to induce spontaneous tumors at the age of >10 months (Levine et al., 2017) (**Fig. 1A**). To accelerate tumor onset we used the irradiation-induced thymic lymphoma mouse model (4x 1.75 Gy low-dose irradiation, 7-day interval) on top (Labi et al., 2010). Low dose irradiation causes leukocyte cell death and activates hematopoietic stem cells (HSC) to re-establish a hematopoietic system. The repeated activation of HSC causes DNA damage and therefore drives mutations in HSC that ultimately drives thymic lymphomas in this model. In the recovery period (4x 7 days) after irradiation mice were set on doxycycline food to induce extra centrosomes to assess whether the PIDDosome is activated *in vivo* to halt malignant transformation. Strikingly, extra centrosomes delay tumor onset in the irradiation-induced tumor model (**Fig. 1B**). Next, we asked

whether this effect is indeed PIDDosome-mediated. Therefore, we crossed the TET-responsive, doxycycline-inducible PLK4-EYFP transgenic mice with PIDDosome-deficient mice (*Pidd1*$^{-/-}$, *Raidd*$^{-/-}$ or *Caspase-2*$^{-/-}$) and repeated the experiment.

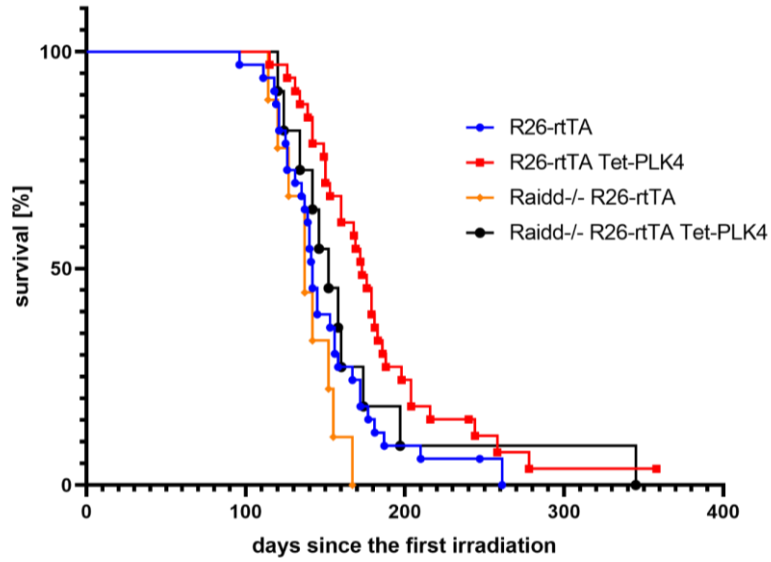

Fig. 2 The PIDDosome halts malignant transformation upon induction of extra centrosomes in an IR-induced thymic lymphoma model *R26-rtTA Tet-PLK4* mice (red) have a delayed tumor onset as compared to littermate control *R26-rtTA* mice (blue). *Raidd*$^{-/-}$ *R26-rtTA Tet-PLK4* mice (black) develop tumors in a comparable range as compared to *Raidd*$^{-/-}$ *R26-rtTA* control mice (yellow).

In line with our hypothesis, extra centrosomes induced by doxycycline do not delay tumor onset in mice deficient for components of the PIDDosome as demonstrated here on a Raidd-deficient background (**Fig. 2**). Data gathered from preliminary analysis of *Pidd1*$^{-/-}$ *R26-rtTA Tet-PLK4* mice so far confirm the barrier function of the PIDDosome in this tumor model, yet the Caspase-2-deficient cohort is only set up recently and it is too early to draw any conclusions here. Nevertheless, these data strongly suggest that the PIDDosome gets activated in response to supernumerary centrosomes *in vivo* to (at least) delay tumor onset.

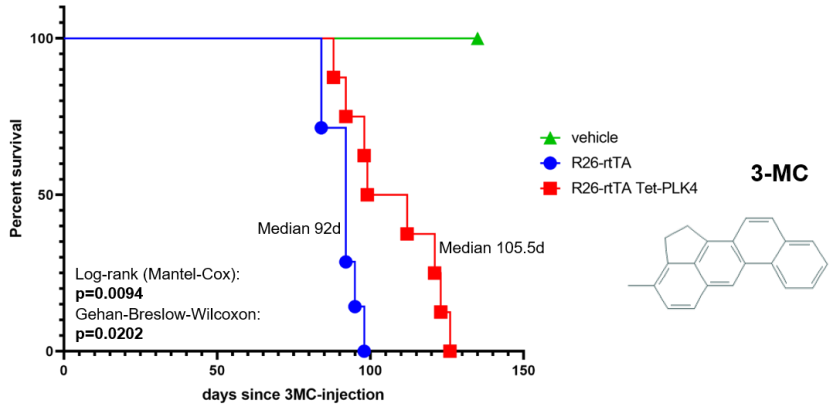

Fig. 3 Extra centrosome induced delay in 3-MC driven sarcomas R26-rtTA Tet-PLK4 mice (red) have a delayed tumor onset as compared to littermate control R26-rtTA mice (blue) in a 3-methyl colanthren (3-MC) induced sarcoma model.

Intra-muscular injection of 3-methyl colanthrene (3-MC) is a well-established mouse sarcoma model. To confirm the findings gathered from the irradiation-induced thymic lymphoma model (**Fig. 1, 2**), we wanted to repeat the experiments using a completely different system *i.e.* the 3-MC sarcoma model. So far, we can report that, in line with the irradiation-induced thymic lymphoma model, also in the 3-MC sarcoma model induction of extra centrosomes results in a delayed tumor onset (**Fig. 3**). This would indicate that also in a sarcoma model the PIDDosome gets activated in response to supernumerary centrosomes *in vivo* to delay tumor onset. However, at the moment it is too early to draw any hasty conclusions as the PIDDosome deficient 3-MC cohort is only set up recently.

Outlook and Discussion

Within this project financed by the Austrian Cancer Society Tyrol we could demonstrate that extra centrosomes can act tumor suppressive when combined with another tumor-driving force *i.e.* DNA damage induced thymic lymphoma or 3-MC induced sarcoma. Although not confirmed yet in the 3-MC model, the data generated so far with the IR-induced tumor model clearly indicate that the PIDDosome is specifically activated in response to supernumerary centrosomes to halt malignant transformation *in vivo* in mice. To assess the level of aneuploidy which can be expected in such tumors arising from mice overexpressing PLK4 we sent tumor samples to our collaborator in Groningen, NL, Floris Foijer (ERIBA Laboratory of Genomic Instability in Development and Disease) for low coverage chromosome copy number analysis of single cells via sequencing.

Zusammenfassung

Mit diesem von der Krebshilfe-Tirol ermöglichten Projekt konnten wir zeigen, dass das PIDDosome (**Fig. 4**) unter bestimmten Umständen eine Barriere für Krebsentstehung sein kann. Im Konkreten konnten wir in zwei verschiedenen Tumormodellen – Strahlungsinduziertes Thymuslymphom und Karzinogen (3-MC) induziertes Sarkom – in Mäusen beobachten, dass die Tumorentstehung verzögert ist, wenn eine Zelle zu viele Zentrosomen enthält. Ein zu viel an Zentrosomen fördert im Normalfall das ungerechte Aufteilen von Chromosomen (Aneuploidie) während der Zellteilung und es wurde bereits gezeigt, dass dies onkogen wirkt, dass also dadurch Tumore entstehen können (Levine et al., 2017). Mit unseren Versuchen konnten wir hier nun zeigen, dass ein zu viel an Zentrosomen auch tumorsuppressiv wirken kann, also die Tumorentstehung hemmt, wenn schon eine andere Tumor-treibende Kraft (Strahlungs- oder Karzinogen-induzierte DNA-Schäden und daraus folgende Mutationen) in einer Zelle vorhanden ist. Weiters können wir zumindest vorläufig sagen, dass das PIDDosome hierbei eine wesentliche tumorsuppressive Funktion hat.

Die Erkenntnisse aus dieser Arbeit könnten helfen neue Chemotherapeutika zu entwickeln und sogenannte Tumormarker-Gene zu definieren. Weiters wollen wir mit unserer Arbeit auf den Nutzen, aber auch auf das mögliche Risiko von potenziellen PIDDosome-Inhibitoren aufmerksam machen. Letztlich wird auch diese Arbeit dazu beitragen Krankheiten zu bewältigen, welche durch deregulierte Zentrosomen- und Chromosomenzahl verursacht werden, wie beispielsweise Krebs.

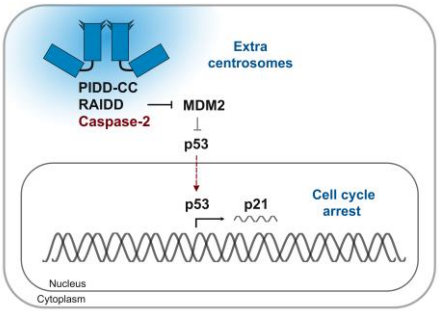

Fig. 4 PIDDosome Aktivierung aufgrund von zu vielen Zentrosomen In einem noch nicht genau bekannten Mechanismus „zählt" PIDD1 Zentrosomen. Befindet sich mehr als ein Zentrosom in einer Zelle, formiert sich das PIDDosome, um Caspase-2 zu aktivieren. Caspase-2 schneidet folglich MDM2 was wiederum zur Stabilisierung des Tumorsuppressors p53 führt und in einem p21-abhängigen Zellzyklusarrest resultiert (Sladky et al., 2017).

References / Referenzen

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