Molecular mechanisms regulated by the histone methyltransferase EZH2 in Adrenocortical carcinoma

Houda Tabbal, Coralie Drelon, Mickael Mathieu, Stéphanie Rodriguez, Amandine Septier, Marie Batisse-Lignier, Typhanie Dumontet, Isabelle Sahut-Barnola, Jean-Christophe Pointud, Anne-Marie Lefrançois-Martinez, Thomas J. Giordano, Enzo Lalli, Bruno Ragazzon, Jérôme Bertherat, Antoine Martinez & Pierre Val.

Introduction

Adrenocortical carcinoma (ACC) is a highly aggressive cancer. Deregulation of epigenetic factors is frequently involved in tumour progression. We have recently shown that the histone methyltransferase EZH2 is the most deregulated epigenetic histone modifier in ACC (Drelon et al., Hum Mol Genet 2016)**.**

Expression of the pro-apoptotic factor NOV/CCN3 is decreased in ACC. In prostate cancer, NOV expression is inhibited by the androgen receptor, through recruitment of EZH2 and deposition of the H3K27me3 mark.

NOV has previously been identified as a negative target of the nuclear receptor SF1 in ACC cells. Our hypothesis is that EZH2 inhibits NOV expression in ACC, through interaction with SF1. This may favour malignant progression in ACC by inhibition of key apoptosis stimulators.

Context of the study

We are currently conducting a combination of transfection and ChIP experiments to evaluate a cooperation between SF1 and EZH2 to repress NOV expression and block apoptosis in ACC.

Results

NOV is downregulated in ACC compared with normal adrenals and adrenocortical carcinomas in two cohorts

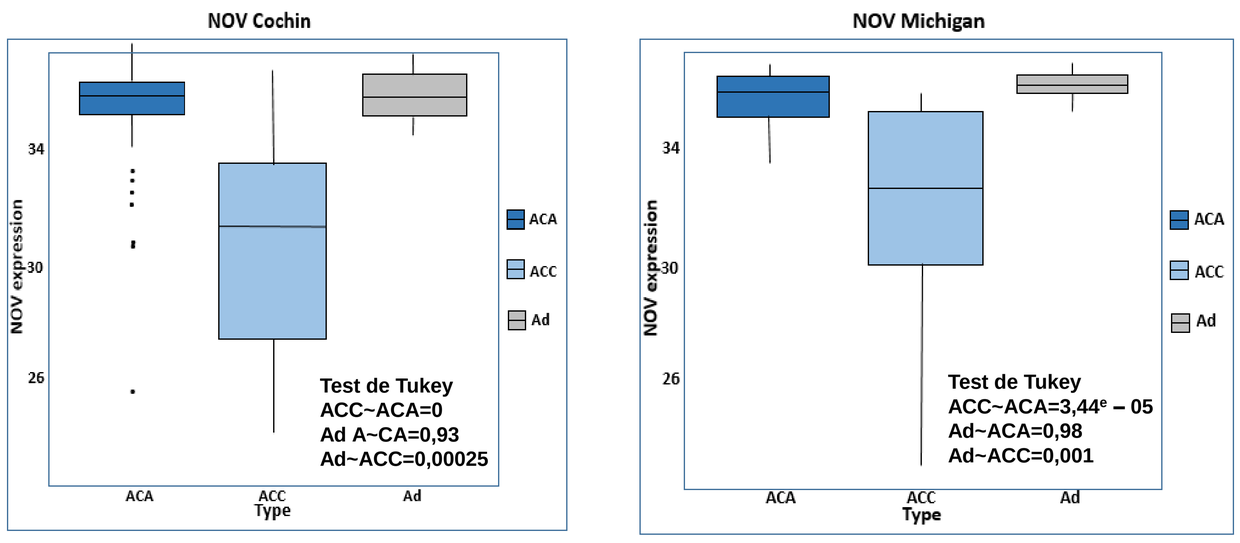


Figure1: Expression levels of NOV were evaluated in patients from the Michigan and Cochin cohorts. Expression levels in ACC were compared with normal adrenals and adrenocortical adenomas and significance of variation was evaluated by ANOVA followed by Tukey test in both cohorts

NOV expression is inversely correlated with expression of EZH2 in humans

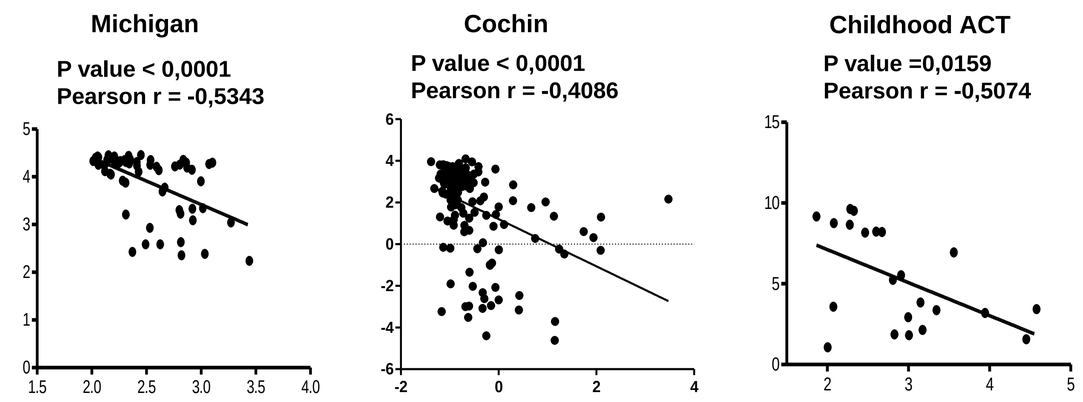


Figure2: Relative expression levels of NOV and EZH2 were extracted from Michigan and Cochin cohorts of adult ACC and from childhood ACT (Pinto et al. Nat Comms 2015). A negative correlation was observed between the two actors in the three cohorts. This suggests that EZH2 is a negative regulator of NOV.

NOV is overexpressed in EZH2 KO mice

Figure3: Relative expression levels of NOV are higher in EZH2 KO mice compared with the wild-type. This suggests that absence of EZH2 resulted in an upregulation of NOV.

EZH2 inhibits NOV in H295R cells

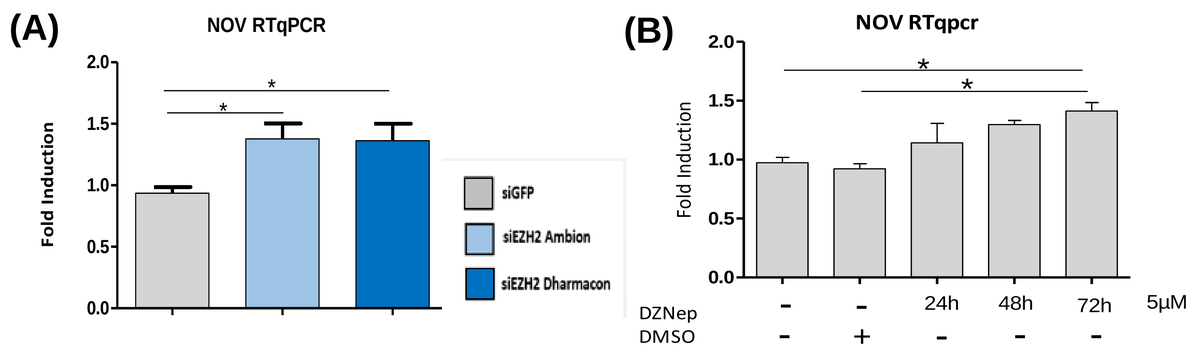
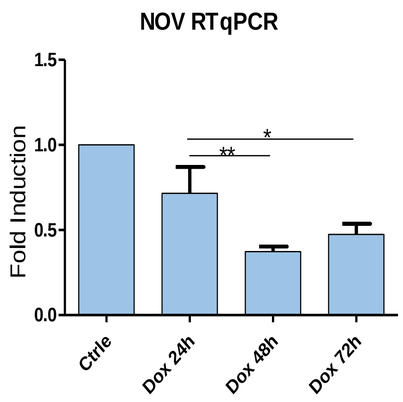


Figure4: siRNA mediated knock down of EZH2 (A) and its pharmacological inhibition with DZNep (B), induce overexpression of NOV.

NOV production is negatively regulated by SF1 overexpression in H295R TR-SF1 cells



H295R TR-SF1 is a cell line that overexpresses SF1 in response to Doxycycline (Doghman M, JCEM 2007)

Figure5: NOV mRNA accumulation levels are decreased in H295R TR-SF1 cells in response to doxycycline treatment

NOV negative regulation by SF1 is abrogated in the absence of EZH2 in H295R TR-SF1 cells

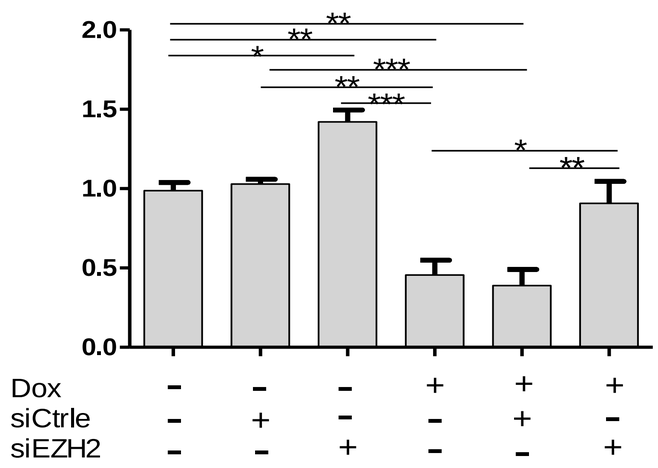
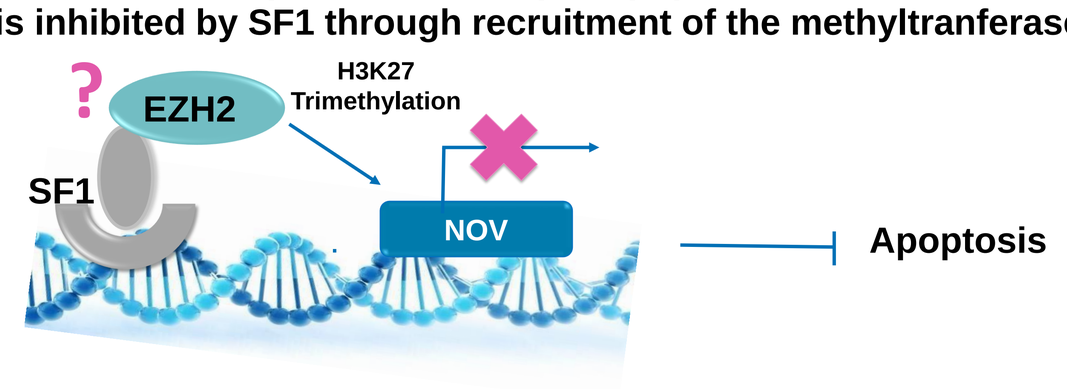


Figure6: NOV mRNA accumulation levels are restored by knockdown of EZH2 in H295R TR-SF1 cells treated with doxycyclin. NOV mRNA accumulation levels are no longer affected by SF1 overexpression when EZH2 mRNA levels levels are down-regulated by siRNA

Conclusion

It seems that expression of the pro-apoptotic factor NOV/CCN3 is inhibited by SF1 through recruitment of the methyltranferase EZH2.



Perspectives

- **Inhibition of NOV using → anti-NOV antibody / siNOV**
- **ChIP and co-immunoprecipitation of EZH2 and SF1 to further understand regulation of NOV expression**

FONDATION ARC POUR LA RECHERCHE SUR LE CANCER Reconnue d'utilité publique
Instituts thématiques Inserm Institut national de la santé et de la recherche médicale
cnrs
CLERMONT UNIVERSITÉ
GReD GENETIQUE REPRODUCTION & DEVELOPPEMENT
ESFE