ROLE OF TANKYRASE 1/2 IN THE HYPOXIC RESPONSE

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BACKGROUND: **Tankyrase 1** (TNKS1) and **Tankyrase 2** (TNKS2) are two proteins that form a distinct subgroup inside the PARP family. Both TNKS share 82% of its sequence and have been linked to different cellular functions such as mitotic progression, glucose metabolism, stress granule formation and Wnt signaling. Furthermore, altered levels of TNKS1 and/or TNKS2 expression have been reported in several types of cancer such as colon, lung or brain. Both tankyrases synthesize linear chains of poly(ADP-ribose) (PAR) to produce posttranslational modifications of their target proteins and also itself through automodification. PARylation by TNKS appears to be tightly linked to ubiquitination by ubiquitin E3 ligases like RNF146. Deficient angiogenesis leads to tumor hypoxia resulting in increased aggressiveness and therapeutic resistance. The adaptation to this situation is carried out by the heterodimeric transcription factors hypoxia-inducible factor (HIF). In particular, the oxygen-dependent protein HIF-1α/HIF-2α and the constitutively expressed protein HIF-1β are responsible for the induction of genes that allow the adaptation and survival of cells to hypoxia.

AIM: Previous results from our group have shown that the posttranslational modification by PARylation is needed for the optimal activation of HIF-mediated response to hypoxia, affecting both protein stability and transcriptional activation. In view of the pivotal role of TNKS-derived poly(ADP-ribose) synthesis in the control of protein stability, we aimed to elucidate the implication of TNKS in the regulation of HIF-1α turnover and function in different tumor settings.

FIG 1. TNKS1/2 STRUCTURE, FUNCTIONS AND PROTEIN DEGRADATION MECHANISM

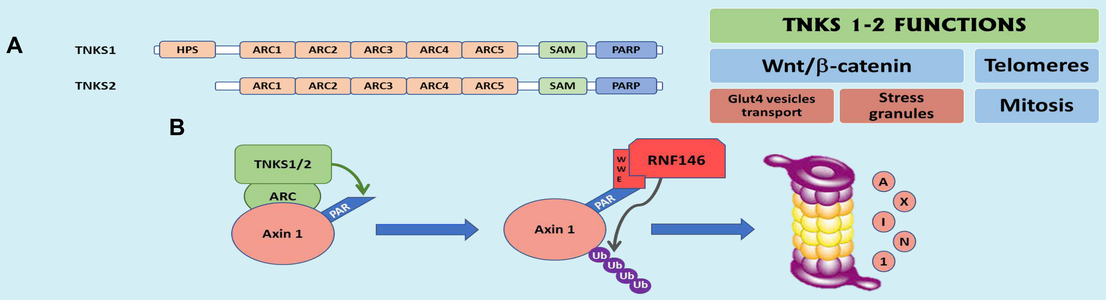

FIG 1. (A) TNKS1/2 structure and functions. Both tankyrases have a similar structure which contains a conserved PARP signature motif inside the C-terminus that catalyzes the synthesis and transfer of Poly-(ADP-ribose) to target proteins. There is also an sterile alpha motif that is implicated in the formation of homo- and heterooligomers and five ankyrin repeat clusters that allow protein-protein interactions. TNKS1 also present a His, Pro and Ser rich region at the N-terminus which function is not well-known. **(B) Protein degradation mechanism by TNKS1/2**. Tankyrase 1/2 can add chains of linear PAR to their substrates. Then, the ubiquitin E3 ligase RNF146 is able to recognize the linear PAR due to its WWE domain and synthesize a Lys48-linked polyubiquitin chain that lead the protein to degradation via proteasome. This mechanism is well-known, but there are other ubiquitin links involving Lys11, Lys27 or Lys63 that trigger other biological responses like disruption of complexes, endosomal sorting or lysosomal degradation.

FIG 2. INTERACTION BETWEEN HIF-1α AND TNKS1/2 IN HELA CELLS

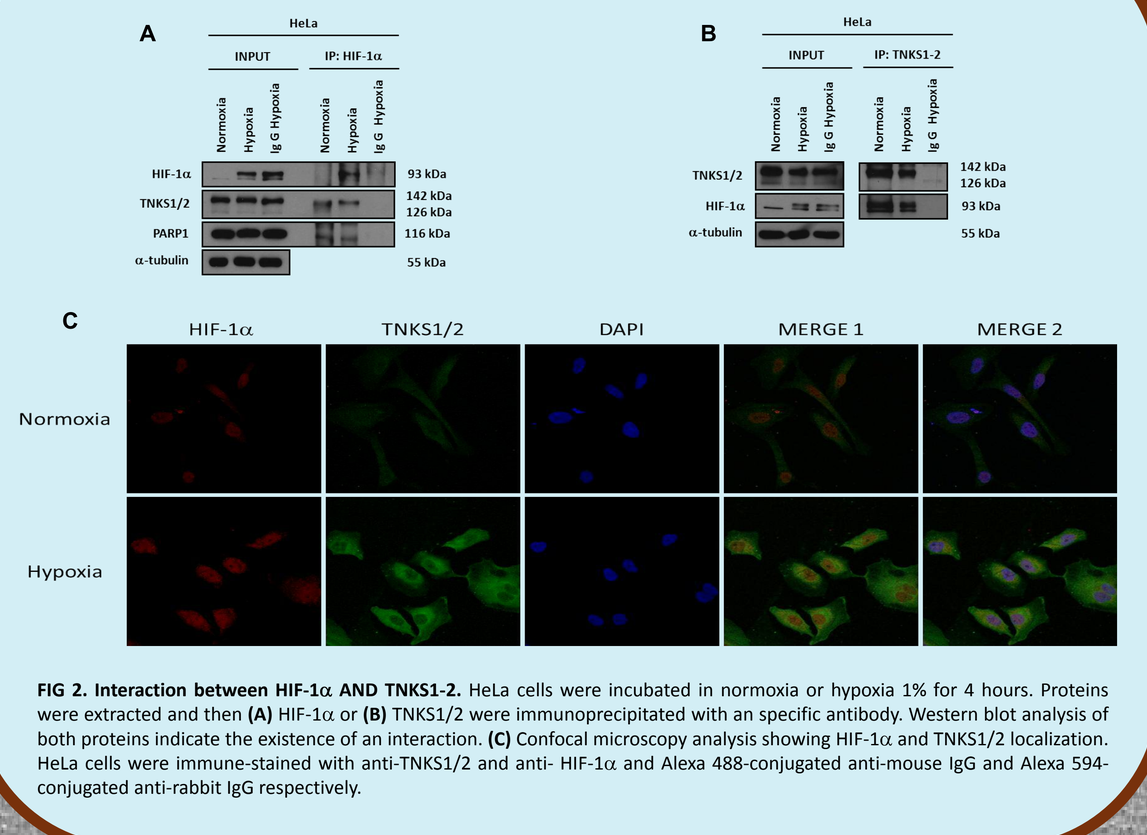

FIG 2. Interaction between HIF-1α AND TNKS1-2. HeLa cells were incubated in normoxia or hypoxia 1% for 4 hours. Proteins were extracted and then **(A)** HIF-1α or **(B)** TNKS1/2 were immunoprecipitated with an specific antibody. Western blot analysis of both proteins indicate the existence of an interaction. **(C)** Confocal microscopy analysis showing HIF-1α and TNKS1/2 localization. HeLa cells were immune-stained with anti-TNKS1/2 and anti- HIF-1α and Alexa 488-conjugated anti-mouse IgG and Alexa 594-conjugated anti-rabbit IgG respectively.

FIG 3. LOSS OF HIF-1α PROTEIN STABILITY AND RELATED-GENE GLUT1 mRNA AFTER TNKS1/2 INHIBITION WITH XAV939 IN HEK-293T AND MUM2B CELLS

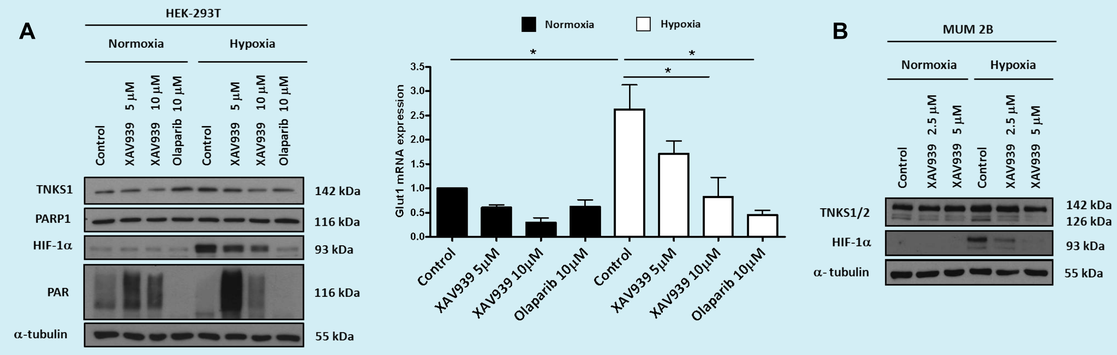

FIG 3. Western blot analysis of HIF-1α protein and related-gene Glut1 mRNA expression levels at different doses of TNKS1/2 inhibitor XAV939. (A) HEK-293T and **(B)** MUM 2B cells were treated with XAV939 during 24 hours (2.5 μM, 5 μM and 10 μM), and incubated in normoxia or hypoxia 1% for 4 hours. PARP1 inhibitor Olaparib was used as a positive control. HIF-1α protein was measured by Western blot and Glut1 mRNA gene expression was evaluated by qPCR and both levels decrease with XAV939 in a dose dependent manner.

FIG 4. LOSS OF HIF-1α PROTEIN STABILITY AFTER TNKS1/2 SILENCING WITH siRNA IN HEK-293T AND HELA CELLS

FIG 4. Western blot analysis of HIF-1α expression after TNKS1/2 silencing with siRNA. (A) HEK-293T and **(B)** HeLa cells were transfected with TNKS1 (60 nM) and/or TNKS2 (30 nM) siRNA during 48 hours, and incubated in normoxia or hypoxia 1% for 4 hours. HIF-1α was measured by Western blot and protein levels decrease with the loss of TNKS1/2.

FIG 5. LOSS OF HIF-1α PROTEIN STABILITY AFTER TNKS1/2 INHIBITION WITH XAV939 AND G007-LK DURING NORMOXIA IN HEK-293T

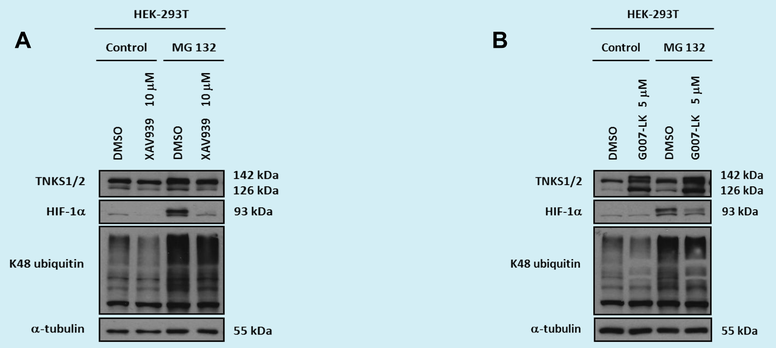

FIG 5. Western blot analysis of HIF-1α protein with different TNKS1/2 inhibitors XAV939 and G007-LK during normoxia. HEK-293T cells were treated with XAV939 10 μM **(A)** or G007-LK 5 μM **(B)** during 24 hours and incubated in normoxia with or without a proteasome inhibitor MG132 3 μM for 4 hours. HIF-1α protein was measured by Western blot and protein levels decrease with TNKS1/2 inhibition despite proteasome inhibition.

CONCLUSIONS:

- Our experiments suggest that there is a connection between TNKS1/2 and HIF-1α, since TNKS inhibition or silencing TNKS1/2 results in HIF-1α decreased stability and transcriptional activation.
- Immunoprecipitation results also hint at the formation of a complex between TNKS and HIF-1α, which is even stronger during normoxia.
- PARylation by tankyrases is implicated in HIF-1α stability and transcriptional activation capacity.

FUTURE REMARKS

- **Generation and analysis of TNKS1, TNKS2 and TNKS1/2 CRISPR-CAS9 models**
- **Test the effect of TNKS1/2 inhibition in colon cell lines**
- **Perform PARylation in vitro assays with wild type versus mutant HIF-1α**

REFERENCES

- **Haiakarainen T et al, *Curr Pharm Des* 2014**
- **Bhardwaj A et al, *Nat Commun* 2017**
- **Li N et al , *Nat Commun* 2019**

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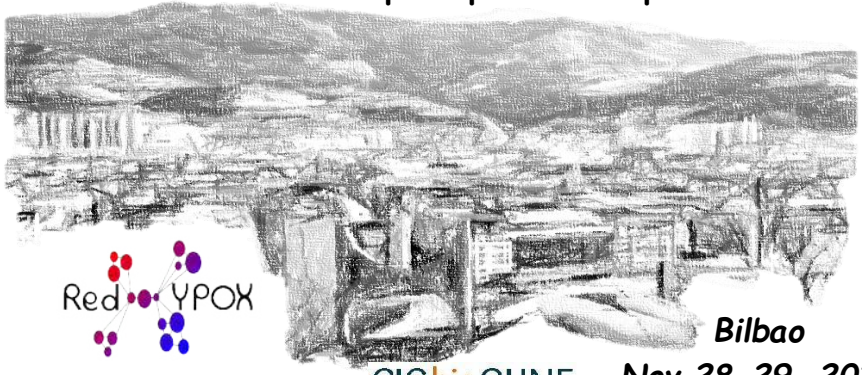

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November 28, 2019 (Thursday)

11:00-11:45: Registration & poster setup

11:45:12:00: Welcome words

12:00-13:00: Keynote by **Sonia Rocha** (University of Liverpool)

How does chromatin react to hypoxia?

13:00-14:30: Lunch and poster view

14:30-16:00: Session I:

(Chair: Antonio Martínez)

Cristina Rodríguez (Instituto de Investigación Biomédica de Salamanca)
Brain neovascularization determines functional recovery after intracerebral hemorrhage through the p53 signaling pathway

María Isabel Álvarez (Instituto de Biomedicina de Sevilla)
Non-productive angiogenesis disassembles Aß plaque blood vessels limiting local clearance

Benilde Jimenez Cuenca (Instituto de Investigaciones Biomédicas "Alberto Sols")
Angiogenesis and hypoxia: understanding endothelial cell proliferation control

Mónica Fernández Cortés (Instituto de Parasitología y Biomedicina López Neira)
PARP inhibition and hypoxia enhance the endothelial phenotype in melanoma cells during vasculogenic mimicry

16:00-16:30: Coffee break

16:30-17:00: Keynote by **Mariona Graupera** (IDIBEL)

PI3King in blood vessels: the importance of keeping PIP3 levels in shape

17:00-18:00: Session II:

(Chair: Juan Pedro Bolaños)

Lin Gao (Instituto de Biomedicina de Sevilla)
Acute O_2 sensing through HIF2a-dependent expression of atypical cytochrome oxidase subunits in arterial chemoreceptors

Stefan Hümmer (Instituto de Investigación Vall d'Hebron)
Intercellular communication in the tumour environment

Lucía Celada (Instituto de Investigación Sanitaria del Principado de Asturias)
Hypoxia and pseudohypoxia in cancer: integrated analysis of TCGA databases

18:00-18:30: Poster storm

(Chair: Teresa Martín-Mateos)

21:15: Dinner

November 29, 2019 (Friday)

9:00-10:00: Through creating the optimal hypoxia environment by Baker/Ruskinn, and Biospherix

(Chair: María Calzada)

10:00-11:30: Session III:

(Chair: Mariló Chiara)

Julián Aragonés (Hospital Universitario Santa Cristina)
Regulation of oxidative and reductive glutamine metabolism executed by HIF1

Agueda González-Rodríguez (Hospital Universitario Santa Cristina)
Hypoxia and non-alcoholic fatty liver disease

Javier Sevilla (Hospital Universitario Santa Cristina)
Molecular mechanisms involved in COPD development beyond hypoxia

Lucía Fadón (CIC bioGUNE/CIC bioMAGUNE)
Effects of 2-Deoxyglucose on the Sugen/Hypoxia Rat Model of Pulmonary Arterial Hypertension

11:30-12:00: Coffee break

12:00-13:30: Session IV:

(Chair: Alberto Pascual)

Carmen Choya-Foces (IIS Hospital Universitario de la Princesa)
Unexpected mitochondrial Na+ and Ca2+ movements control mitochondrial redox signalling in acute hypoxia

Rosario Morrugares Carmona (Instituto Maimonides de Investigación Biomédica de Córdoba)
Target identification and mechanism of action of new hypoximimetic compounds

Paloma Narros Fernández (IIS Hospital Universitario de la Princesa)
Role of the mitochondrial Na+/Ca2+ exchanger in the TLR4-HIF1α-mediated metabolic immunomodulation of M1 macrophages

Asís Palazón (CIC bioGUNE)
Role of HIF in anti-tumor immune responses and early development of a small molecule inhibitor of "Factor Inhibiting HIF" (FIH)

13:30-14:00: Concluding remarks

14:00: Lunch and poster viewing