ELSEVIER

Blood 142 (2023) 5659–5661

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Hnrnpk Overexpression Drives Nucleolar Aberrancies Causing Ribosomopathies

Pedro Aguilar-Garrido[1], *Maria Velasco-Estevez, PhD*[2], *Miguel Ángel Navarro-Aguadero*[3], *María Hernández-Sánchez*[4], *Marta Ibáñez-Navarro*[5], *Prerna Malaney, PhD*[6], *Marisa Aitken, BS*[7], *Xiaorui Zhang*[8], *Adrián Fernández*[3], *Alejandra Ortiz-Ruiz*[3], *Álvaro Otero-Sobrino*[3], *Diego Megías*[9], *Manuel Pérez*[1], *Jesús Gómez*[1], *Gadea Mata*[10], *Orlando Domínguez*[1], *Osvaldo Graña*[11], *Pilar Ximénez*[1], *Marta Isasa*[1], *Paloma Jimena-de Andrés*[4], *Sandra Rodríguez*[1], *Raúl Torres*[1], *Miguel Lafarga*[12], *Oleksandra Sirozh*[1], *Vanesa Lafarga*[1], *Joaquin Martinez-Lopez, MD PhD*[13], *Sean M. Post, PhD*[14], *Miguel Gallardo, PhD*[3]

[1] Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain
[2] Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, ESP
[3] Hospital 12 de Octubre, Madrid, Spain
[4] Universidad Complutense de Madrid (UCM), Madrid, Spain
[5] Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, ESP
[6] MD Anderson Cancer Centre, Houston, TX
[7] MD Anderson Cancer Center, Houston, TX
[8] MD Anderson Cancer Centre, Texas
[9] Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[10] Universidad de la Rioja, Logroño, Spain
[11] Universidad San Pablo CEU, Madrid, Spain
[12] Universidad de Cantabria, Cantabria, Spain
[13] Hospital Universitario 12 de Octubre, CIBER-ONC CB16/12/00369, CNIO, Madrid, Spain
[14] MD Anderson, Houston, TX

Background: Protein biogenesis is a complex process involving *nucleoli* and ribosomes. Alterations in any step could lead to alterations in ribosome functionality and protein synthesis. Hnrnpk is an RNA-binding protein (RBP) involve in these processes, finding that an overexpression (OE) produces nucleus and nucleolar stress (NS), decreases transcription, and drives an imbalance in ribosome biogenesis, causing a reduced translation.
Aims: To elucidate how hnRNP K dysregulation affects the hematopoietic stem cell (HSCs) biology.
Methods: To study the impact of Hnrnpk OE *in vivo*, we developed an inducible tamoxifen mouse model, *Hnrnpk* $^{Tg/hUbc\text{-}CreERT2}$. Survival was evaluated by Kaplan-Meier, phenotype was described by symptoms/signs, CBC, bone marrow (BM) H/E, IHC and FCM analysis, and serum IL-6 ELISA.
HSCs were cultured to study the impact of Hnrnpk OE in the HSCs dynamics. Hnrnpk OE was established *in vitro* using CRISPR/SAM. RNA-seq analysis was performed in a single read 85-base format and analyzed with DESeq2. TMT-based deep proteome profiling was also performed. Both were GSEA preranked.
Transcription and translation were tested using Click-it RNA and HPG kit respectively, and translation efficiency by polysome assay. NS were analyzed by confocal microscopy and transmission electron microscopy (TEM). Protein-protein interaction between Hnrnpk and Ncl was studied by IP. Possible phenotype rescue was carried using *Hnrnpk* $^{Tg/hUbc\text{-}CreERT2}$/ *c-Myc* $^{lox/wt}$, *Hnrnpk* $^{Tg/hUbc\text{-}CreERT2}$/ *Tp53* $^{lox/wt}$ and *Hnrnpk* $^{Tg/hUBC\text{-}CreERT2}$/ *Ncl* ^{KD} *in vitro* and *in vivo* models. Cell cycle FACS, senescence assays and karyotyping were performed. Molecular mechanism was elucidated by qRT-PCR and WB.
Results: Hnrnpk $^{Tg/hUbc\text{-}CreERT2}$ mice had widespread Hnrnpk OE and lifespan's reduction. By CBC, we found the development of leukopenia, lymphopenia, anaemia and thrombocytopenia (Fig.A). BM H/E, IHC and FCM showed a reduction of B220 $^{+}$ and CD34 $^{+}$ and Sca1 $^{+}$ HSCs, and an increment in myeloid cells (Fig. B). Also, we found higher senescent β-galactosidase expression in BM and IL-6 *in vivo* (Fig.C). Then, we found a decay in viability and an exhaustion in HSCs (Fig.D).
To understand Hnrnpk implication in BM failure phenotype *in vivo*, we generated Hnrnpk OE cells (Fig.E). RNA-seq showed an upregulation in G2/M-checkpoint pathway related molecules (Fig.F), confirmed by FACS analysis, showing and increment of arrested G2/M phase-cells (Fig.G). Moreover, we showed a rise in β-galactosidase activity, polyploidy and genomic instability

(Fig. H), linked to an increment in p21 and p16 (Fig.I). Then, TEM revealed nucleolar alterations in the *in vitro* model, including segregation, anormal accumulations or fragmentation of nucleolar components, alterations validated by confocal microscopy (Fig.J-K). Also, we found a Ncl increment *in vitro*, consistent with the protein-protein interaction between Hnrnpk and Ncl. Then, proteomics showed that Hnrnpk OE correlates with ribosome biogenesis regulators, c-Myc and mTOR dysregulation (Fig.L). Moreover, we found a decrease in transcription (Fig.M), consistent with rRNAs reduction, driving translation and protein synthesis deficiency (Fig.N). There was a partial reversion of NS hallmarks in *Hnrnpk* $^{Tg/hUbc\text{-}CreERT2}$/ *c-Myc* $^{lox/wt}$, *Hnrnpk* $^{Tg/hUbc\text{-}CreERT2}$/ *Tp53* $^{lox/wt}$ and *Hnrnpk* $^{Tg/hUBC\text{-}CreERT2}$/ *Ncl* ^{KD} MEFs *in vitro* models and partially rescue phenotype *in vivo* (Fig.O). Finally, we focused on the nucleolus. Thus, Hnrnpk OE cells showed a reduction in Fbl, increase in Ncl and Ncl diffusion in nucleoplasm. All these data suggest the existence of a ribosompathy-like phenotype.

Conclusions: this work found that RBPs dysregulation such as Hnrnpk OEdrives BM failure phenotype, promoting the exhaustion of HSCs by nucleoulus/ribosome dysregulation that triggers cell cycle arrest and apoptosis, dependent of p53. We therefore suggest that Hnrnpk induces ribosome dysfunction consistent with some types of ribosomopathies.

This work was financially supported by CRIS contra el Cancer Association (NGO) AES ISCIII (PI18/00295, PI21/00191), ISCIII Miguel Servet (CP19/00140), Cancer Research UK [C355/A26819], Sociedad Española de Hematología y Hemoterapia (SEHH), FC AECC and AIRC under the Accelerator Award Program and National Cancer Institutes of Health Award (R01CA207204, SMP) Leukemia and Lymphoma Society (6577-19, SMP).

Disclosures Post: *LinBioscience:* Research Funding.

https://doi.org/10.1182/blood-2023-178022

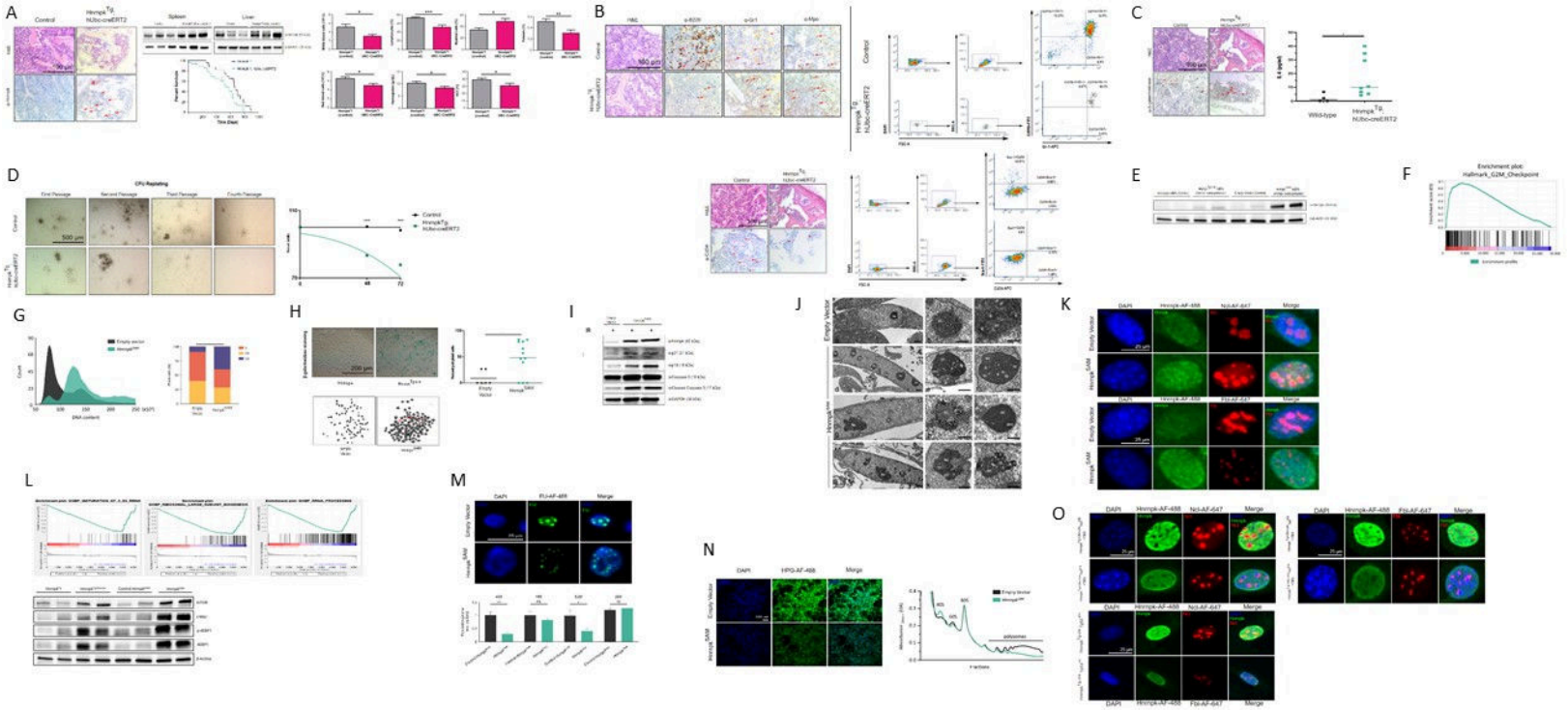

Figure A: H&E and hnRNP K IHC in mice BM; WB showing hnRNP K in liver and spleen of *in vivo* model; CBC: total WBC (p=0.032), % lymphocytes (p=0.001), % myeloid cells (p=0.017) and % platelets (p=0.009), total red blood cells (p=0.016), hematocrit (p=0.050) and hemoblobin (p=0.017) . **Figure B**: H&E and B220, Gr1 and Mpo IHC in mice BM; FCM of total BM, $Cd11b^{+}/Gr\text{-}1^{+}$(p = 0.0327*); H&E and Cd34 IHC in mice BM; FCM of total BM, $Cd34^{+}$ (p = 0.0208*) and $Sca\text{-}1^{+}$ (p = 0.0424*). **Figure C**: H&E and β-galactosidase IHC in mice BM; IL-6 ELISA from $Hnrnpk^{Tg/hUbc\text{-}CreERT2}$ mice (+4-OHT) vs $Hnrnpk^{Tg}$ (+4-OHT). **Figure D**: CFU replating assay of HSCs from total BM; Viability assay of HSCs from total BM. **Figure E**: WB showing hnRNP K OE in *in vitro* models. **Figure F:** RNA-seq analysis shows an upregulation of G2/M checkpoint genes. **Figure G**: hnRNP K overexpressing cells showed an increase in the G2/M population after cell cycle FACS. **Figure H**: Bright-field microscope images of β-galactosidase staining in hnRNP K *in vitro* models; increment of polyploidy; increment of DM chromosomes. **Figure I:** WB showing p16 and p21 in *in vitro* models. **Figure J**: TEM images showing nucleolar alterations in the *in vitro* model, such as segregation, anormal accumulations or fragmentation. **Figure K**: Confocal microscopy showing that the increment in Hnrnpk correlates with an increment in Ncl, its deslocalization to nucleoplasm, reduction in Fbl and nucleolar abnormalities. **Figure L**: TMT analysis shows a dysregulation in pathways related to ribosomal biogenesis; WB showing an increment in c-Myc and mTOR in *in vitro* models. **Figure M**: Confocal microscopy EU assay from *in vitro* models; RT-qPCR showing decrease in rRNAs in *in vitro* models. **Figure N**: Confocal microscopy HPG assay from *in vitro* models; Polysome assay. **Figure O**: Confocal microscopy showing partial rescue of $Hnrnpk^{Tg/hUbc\text{-}CreERT2}/c\text{-}Myc^{lox/wt}$, $Hnrnpk^{Tg/hUbc\text{-}CreERT2}/Tp53^{lox/wt}$ and $Hnrnpk^{Tg/hUBC\text{-}CreERT2}/Ncl^{KD}$ *in vitro* models.

Figure 1