Abstract—Bone Cancer Group, IGM-CNR, UOS Bologna

The bone cancer research group is occupied with determining the underlying causes of pediatric bone cancers, mechanisms of disease progression and the discovery of novel targets for therapeutic intervention. The main areas of research focus on the role of metabolic and mechano-/stress/inflammatory signaling on gene transcription and RNA metabolism during tumor progression and metastases.

Altered Lamin expression has been detected in many human cancers. Changes in Lamin expression can alter the rigidity of the nuclear envelope influencing gene expression associated with the nuclear ingress of various transcription factors that promote tumor invasiveness, but also modify the response to mechanical forces. Lamin A plays an essential role in cytoskeletal organization which is important for cell migration. We have demonstrated that alterations in Lamin A expression correlate with malignant transformation in osteosarcoma (OS), consistent with the hypothesis that dysregulation of Lamin A favors the capability of cells to migrate, promoting metastasis and with the known correlation between low type-A Lamin expression and an immature cellular phenotype. Interestingly, expression of the Ewing sarcoma (ES) associate fusion protein EWS-FLI1 results in reduced Lamin A expression. Our future aim is to determine how and why type-A Lamin expression is decreased in OS and ES by examining both gene expression and alternative splicing of the Lamin A/C gene.

The ankyrin-repeat containing domain protein 2 (ANKRD2), a structural protein, is an interactor of type-A Lamins and plays a key role in the transcriptional response to mechanical stress. Analysis of ANKRD2 in OS cells has demonstrated that its overexpression results in diminished proliferation, migration and clonogenicity but enhances attachment-independent growth, while reduced expression of ANKRD2 is associated with inhibited proliferation and clonogenic ability of OS. These effects likely occur through the ability to modulate NF-κB-dependent inflammatory signaling, and this activity can be altered through AKT-dependent modification of ANKRD2 on S99. Our aim in this regard is to examine further NF-κB signaling in OS cells with altered ANKRD2 expression, using real-time PCR, as well as determine the protein:protein interactions of ANKRD2 and ANKRD2 when phosphorylated on S99 (ANKRD2-S99D), using affinity purification-mass spectrometry (AP-MS).

The axis between AKT, the double-strand RNA-dependent kinase, PKR, and the adenosine deaminase acting on double-strand RNA proteins (ADAR1 and ADAR2) is being examined for its role in OS. PKR is a stress/inflammation-activated kinase that is also activated in response to several cytotoxic cytokines (TNFα, IL-1, IL-6) and growth factors (PDGF, FGF, IGF-1) together with AKT. PKR is overexpressed in a majority of OS patients and ectopic overexpression of PKR in OS cell lines resulted in enhanced migration and attachment-independent growth. In contrast, overexpression of DN-PKR had the opposite effect. Interestingly, OS cells express a number of alternatively spliced forms of PKR. In OS patients, expression of full-length PKR is associated with a poor overall survival, while expression of an alternately spliced form of PKR, PKRΔ11, is associated with a favorable overall survival. Moreover, AKT is also recognized as a major regulator in OS. Common nuclear interactors of AKT and PKR are the RNA editase proteins, ADAR1 and ADAR2, whose activity can influence RNA splicing, miRNA targeting and mRNA protein coding. Our group demonstrated that AKT kinases phosphorylate ADAR1 and ADAR2 in the nucleus at a key site in the editase domain, thus altering their editase activity. Our aim is to identify splicing variants of PKR expressed in OS and determine their protein:protein interactors using AP-MS, as well as determine their role in OS cell proliferation, migration, attachment independent growth, and response to therapy. As PKR interacts and its activity can be regulated by ADAR1, we are looking to determine if the AKT-ADAR-PKR axis has a role in promoting alternative splicing of PKR in OS.
Keywords: Sarcoma, RNA metabolism, stress/inflammation

Contacts:

Dr. William Blalock (william.blalock@cnr.it)
Dr. Vittoria Cenni (vittoria.cenni@cnr.it)
Dr. Francesca Chiarini (francesca.chiarini@cnr.it)
Dr. Manuela Piazzi (manuela.piazzi@cnr.it)

Websites:

http://www.igm.cnr.it/pagine-personali/blalock/
http://www.igm.cnr.it/pagine-personali/cenni/
http://www.igm.cnr.it/pagine-personali/chiarini/

Funding:

MIUR-PRIN-2018- 2017RKWNJT (DSB.AD007.195.001)
Leukemia Research Foundation (DSB.AD001.035.001/DSB.AD006.220.001)
AIRC-IG-2015-17137 (DSB.AD006.145.001)