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Cell cycle protein BORA mediates tumor development via modulation of cellular survival and migration pathways

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Introduction: Polo-like kinase 1 (Plk1) is an attractive mitotic target to treat different types of cancer. A variety of anti-Plk1 agents are presently in clinical trials with successful results. Nonetheless, the use of Plk1 ATP-competitive inhibitors have been associated to dose-limiting toxicity and low specificity. Hence, to find alternative strategies to treat Plk1 with less adverse effects is paramount**.** We considered to target the C-terminal part of Plk1 by its main coactivator, BORA *(Aurora Borealis)* since BORA works turning on/off the kinase activity by binding the Plk1-Polo-Box domain. Besides, BORA *per se* has been described as regulator of spindle stability being essential to a proper chromosome segregation. Disorders in genomic integrity generate vulnerabilities that can be exploited therapeutically. Particularly in ovarian cancer (OC), the most lethal gynecology malignancy with a 5-year survival rate of approximately 30%, and with 85% of patients developing resistance to the current treatments, raising the need for alternative treatments.

Objectives: The objectives of the work include (i) Analyze the expression levels of BORA in primary OC tumor samples and correlate its expression with clinical and molecular variables (ii) Provide new insights into the mechanistic role of BORA in cancer by (ii.a) gain of function assays overexpressing BORA and characterizing its implication as oncogenic protein (ii.b) loss of function experiments and exploring whether it could represent a promising novel therapeutic approach. (iii) Develop new strategies to modulate its downstream-function by exploring the combination of clinically relevant inhibitors to manage OC treatment.

Methods*: In silico* data was obtained from The Cancer Genome Atlas (TCGA) database. Human tissue-samples were collected from benign and ovarian cancer patients from the Vall d'Hebron Hospital in Barcelona. *In vitro* studies were carried out using a wide panel of different cancer cell lines with different histology and mutational profile. Pre-clinical *ex vivo* models were stablished by using patient-derived ascitic cells collected at the time of the surgery and grown under anchorage independent conditions*. In vivo* studies were performed by subcutaneous injection of the cells in to NMRI mice. A whole transcriptome analysis was carried out to unveil the pathways downstream of BORA depletion. Clinically relevant inhibitors were used both *in vitro* and in *ex vivo* models.

Results: *In silico* data revealed that BORA is overexpressed in several types of cancer compared to normal tissues, particularly in lung, colorectal and OC correlated with poor patients' outcomes. The mutation rates of BORA ranged from 0 to 4% across various cancer types whereas the mRNA overexpression was observed in up to 20% of some cancer types being the genomic alteration more frequent. Specifically, in OC high expression is related to aggressiveness of the disease, late stages and poor survival. Given the BORA well-reported role in regulating mitosis, expression of BORA is strongly correlated to MIk67 and CIN25 expression, a measure for chromosome instability. To analyze this further, human OC tissue samples showed that BORA is overexpressed compared to benign samples both at mRNA and protein level. In addition, BORA expression is higher in metastasis compared to primary tumors in paired patient-samples. *In vitro*, gain of function experiments in an immortalized epithelial ovarian surface line (IOSE) revealed a BORA oncogenic role by up-regulating proliferation and the migration cell capacities. Also, it promoted loss of contact inhibition forming extremely dense multiple layers with isolated 3D structures and most strikingly, promoted tumor development *in vivo*. Loss of function experiments using both lentiviral particles and CRISPR/cas9 technology, impaired proliferation and colony formation and reduced the migration capacity associated with

a G2/M phase arrest and an increase in the apoptosis rate; being essential for cell viability. *In vivo*, BORA depletion impaired tumor growth development. *Ex vivo,* pre-clinical models using OC patient-derived ascitic cells showed that BORA ablation decreased the tumor-sphere forming capacity and reduced spheroids viability. Gene expression profile revealed that BORA controls the expression of different proteins involved in cell death and survival, metastasis, actin cytoskeleton dynamics and muscle and cardiovascular function. BORA also modulates key elements of oncogenic pathways and the combination of inhibitors targeting these proteins displayed synergistic effects in reducing the viability of OC cells, offering a promising new avenue for OC treatment.

Conclusions: Our results report that (1) BORA promotes malignant cell transformation (2) targeting Plk1 activity through BORA represents a novel approach to treat cancer (3) concomitant inhibition of key oncogenic pathways diminishes OC viability, which may in turn improve current therapies.

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