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651. MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

PYGO2-MDR1 Axis in Multiple Myeloma Patients with 1q21 Amplification As Promising Target to Overcome Carfilzomib Resistance

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Multiple myeloma (MM) is a hematological malignancy characterized by an accumulation of clonal plasma cells (PCs) in the bone marrow (BM). Gain and/or amplification of 1q21 (1q21+) is one of the most frequent secondary cytogenetic abnormalities present in MM patients and in smoldering MM (SMM). The incidence and copy number alteration (CNA) of the 1q21 region increase during the progression and relapse of the disease; in fact, additional copies of 1q21 can be detected in around 40% of newly diagnosed MM (NDMM) and 70% of relapse-refractory MM (RRMM). Recently, 1q21+ has been identified as an adverse prognostic factor in MM patients and its presence has been correlated with a shorter duration of the response in MM patients treated with carfilzomib (CFZ)-based regimens. Thus, the identification of possible target genes in 1q21 region is an emerging unmet medical need in MM patients.

PYGO2 (*PYGO2*) is a gene located in the 1q21 chromosomal region and it is a downstream target of β -catenin, and it has been shown to promote the transcription of gene target of the Wnt-signaling, forming a nuclear complex with β -catenin/BCL9. Different studies have reported that upregulation of *PYGO2* is involved in tumorigenesis in breast and lung cancers, showing that overexpression of *PYGO2* leads to drug resistance by promoting the activation of the multidrug resistance 1 polypeptide (*MDR1*).

The expression profile of *PYGO2* in 1q21+ MM patients and its possible role in CFZ resistance is still unknown and have been investigated in this study.

Firstly, we purified CD138⁺ PCs from BM samples from 18 NDMM and from 11 SMM patients. Fluorescent in situ hybridization (FISH) analysis was performed on purified CD138⁺ PCs for all patients to access CNA in the region 1q21. A score indicating the number of 1q21 copies was calculated based on the FISH hybridization pattern of each patient. 14/29 (48%) patients presented CNA in the 1q21 region. The expression profile of all 29 samples was generated using GeneChip ClariomD Arrays (Affymetrix Inc., Santa Clara, CA, USA). The sam package was used to identify differentially expressed genes between 1q21+ and control samples.

Our analysis identified *PYGO2* to be significantly upregulated in patients with 1q21+ when compared with controls ($p=0.0008$). Additionally, we found a positive correlation between gene expression and the 1q21 copy number ($p<0.0001$, $r=0.6738$). Secondly, we evaluated *PYGO2* mRNA expression levels in several human myeloma cell lines (HMCLs) with 1q21 CNA. Our results showed that *PYGO2* is overexpressed in HMCLs with 1q21 CNA. Previous studies in solid tumor have shown that *PYGO2* is upregulated in drug resistant cancer cells, and this upregulation results in overexpression of *MDR1*; indeed, we evaluated the mRNA expression of *PYGO2* in CFZ-resistant HMCLs. Interestingly our results showed that both *PYGO2* and *MDR1* expression are upregulated in CFZ-R cells. We next examined the relationship between *PYGO2* and *MDR1*. We generated a *PYGO2*-knockdown in JJJ3 MM cell line using short hairpin RNA (shRNA) lentivectors. Knockdown of *PYGO2* resulted in a significant decrease in expression *PYGO2*, a decrease in cell viability, and a reduction in *MDR1* expression.

In conclusion, our results show that *PYGO2* expression is significantly upregulated in MM patients carrying 1q21+ and that its expression is correlated with 1q21 copy number. Moreover, we found that the *PYGO2-MDR1* axis is involved in the CFZ resistance in HMCLs MM cells suggesting that *PYGO2* could be a possible future druggable target in 1q21+ MM patients.

Disclosures Giuliani: *DYNAMICON EDUCATION S.R.L.*: Other: PRECEPTORSHIP; *PREX S.R.L.*: Other: PRECEPTORSHIP; *FIRST CLASS EVENTS 6 CONFERENCE*: Other: PRECEPTORSHIP; *VYVAMED*: Other: PRECEPTORSHIP; *ER CONGRESSI*: Other: PRECEPTORSHIP; *EFFETTI S.R.L.*: Other: PRECEPTORSHIP; *BRISTOL MAYERS SQUIBB*: Consultancy; *TAKEDA*: Membership on an entity's Board of Directors or advisory committees; *AMGEN*: Membership on an entity's Board of Directors or advisory committees; *European Myeloma Network*: Research Funding; *PFIZER*: Research Funding; *RAY HEALTHCARE COMMUNICATION S.R.L.*: Other: PRECEPTORSHIP.

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