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Poster





P113

Jay Amin Hydroxamic Acid (JAHA), a histone deacetylase inhibitor with cytotoxic activity and the property to increase DNA repair of triple-negative MDA-MB231 breast cancer cells

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Jay Amin Hydroxamic Acid (JAHA; N8-ferrocenylN1-hydroxy-octanediamide) is a ferrocene-containing analogue of the histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA). JAHA's cytotoxic activity on MDA-MB231 triple negative breast cancer (TNBC) cells at 72 h has been previously demonstrated with an IC₅₀ of 8.45 μM. JAHA's lethal effect was found linked to perturbations of cell cycle, mitochondrial activity, signal transduction and autophagy mechanisms. In order to glean novel insights on how MDA-MB231 breast cancer cells respond to the cytotoxic effect induced by JAHA, and to compare the biological effect with the related compound SAHA, we have employed a combination of differential display-PCR, proteome analysis and COMET assay techniques and shown some differences in the molecular signature profiles induced by exposure to either HDACis. In particular, in contrast to the more numerous and diversified changes induced by SAHA, JAHA has shown a more selective impact on expression of molecular signatures involved in anti-oxidant activity and DNA repair. Besides expanding the biological knowledge of the effect exerted by the modifications in compound structures on cell phenotype, the molecular elements put in evidence in our study may provide promising targets for therapeutic interventions on TNBCs.

P114

NIPBL, a new player with NPMc+ in the onset of Acute Myeloid Leukemia

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BACKGROUND: cohesins form a multimeric protein complex (SMC1A, SMC3, RAD21, STAG and additional proteins NIPBL, MAU2, ESCO1, HDAC8) involved in the cohesion of sister chromatids, post-replicative DNA repair and transcriptional regulation. Recently, recurrent somatic mutations and deletions of cohesins have been reported in the 9% of patients affected by Acute Myeloid Leukemia (AML) and other myeloid neoplasms, suggesting a role for the cohesin-complex in the pathogenesis of AML. Frequently, mutations in cohesin genes co-occurred with the known AML-associated gene nucleophosmin (NPM1) that, when mutated, aberrantly relocates to the cytoplasm (NPMc+). Forced NPMc+ expression in zebrafish causes an expansion of hematopoietic stem cells (HSCs) in line with AML patient features.**METHODS:** Whole-Mount In Situ Hybridization (WISH) and quantitative real time PCR (qRT-PCR) techniques have been used to analyze the expression of markers of different hematopoietic populations (tal1, lmo2, spi1b and mpx) in nipbl-loss-of-function zebrafish embryos. The functional interaction of the genes has been demonstrated with rescue experiment by means of the injection of different zebrafish transcripts.**RESULTS:** in our cohort of adult AML patients we observed a specific reduction in the expression of NIPBL when NPM1 is mutated. Therefore, we generated a zebrafish model with nipbl haploinsufficiency to investigate the hematopoietic phenotype and the interaction between NPMc+ and nipbl in nipbl-loss-of-function zebrafish embryos, we observed an increase in undifferentiated myeloid cells, a phenotype resembling the NPMc+ zebrafish model. Therefore, we investigated a functional interaction between NPMc+ and NIPBL in the onset of the aberrant hematopoietic phenotype in zebrafish and the involvement of the canonical Wnt pathway in this process.**CONCLUSIONS:** we showed for the first time a role for NIPBL during zebrafish hematopoiesis. Our data suggest that its altered expression observed in AML patients and the co-occurrence with NPM1 mutations, might play a role in leukemia onset.

P115

Nuova mutazione di NIPBL nel primo caso di un paziente pediatrico affetto da sindrome di Cornelia De Lange e Leucemia Linfoblastica Acuta

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La Sindrome di Cornelia de Lange (CdLS) è una malattia genetica rara caratterizzata da ritardo di crescita pre- e post-natale, ritardo mentale, dismorfismi del volto e anomalie degli arti superiori. Principali responsabili della malattia sono mutazioni nei geni NIPBL, SMC1A, SMC3, HDAC8 e RAD21, che codificano proteine del complesso delle coesine o associate ad esso. NIPBL è coinvolto in circa il 55% dei casi di CdLS, mentre le altre coesine nel 5%. Mutazioni nei geni delle coesine sono state recentemente identificate in AML, CML e sindromi mielodisplastiche. Nel presente studio riportiamo la descrizione del primo caso di paziente pediatrico affetto da CdLS che ha sviluppato leucemia linfoblastica acuta a precursori B (BCP-ALL). Tramite analisi NGS (RNA Trusight PanCancer, Illumina) su campione di RNA derivato da cellule mononucleate di midollo osseo all'esordio di leucemia, abbiamo identificato due varianti in geni delle coesine, in particolare nel 5 UTR di SMC1A (cromosoma X in paziente maschio) e nell'esone 46 di NIPBL (cromosoma 5, in eterozigosi). La variante di SMC1A rs1264011 non è patogenetica ed è stata confermata sul DNA del paziente sia in remissione di malattia che in campione di mucosa bucale, della madre e del fratello. La variante di NIPBL è una nuova mutazione che causa frameshift. Tale anomalia è stata confermata sul DNA di midollo osseo del paziente sia alla diagnosi che in remissione di leucemia che in campione di mucosa bucale. Entrambi i genitori ed il fratello sono negativi, fenotipicamente normali e non affetti da patologie ematologiche. Mediante NGS è stata identificata una mutazione germinale di TP53 exon4, rs1042522, nota come benigna e coinvolta in meccanismi di resistenza a chemioterapici. Tale mutazione è presente in omozigosi anche nella madre. Inoltre abbiamo identificato due mutazioni in eterozigosi di JAK3 nell'esone 1 (condivisa esclusivamente con il padre) e nell'esone 16 (condivisa con tutta famiglia in eterozigosi), rispettivamente annotate come rs7254346 (benigna) e rs3213409 (nota come benigna se germinale e somatica in ALL e AML). Il ruolo nella leucemogenesi della nuova mutazione del gene NIPBL, identificata nel primo paziente pediatrico CdLS con BCP-ALL, merita ulteriori indagini.

P116

Constitutional loss of function variants in breast cancer patients with a very early age at diagnosis or a previous childhood/juvenile cancer

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Occurrence of neoplastic diseases at very early age is an indicator of genetic predisposition to cancer, even in the absence of a positive family history. To investigate the role of genetic factors in cancers diagnosed at a very early age (<22 yrs), a total of 25 patients were selected from the registry of the Unit of Medical Genetics of the Istituto Nazionale dei Tumori. These included six patients with a diagnosis of breast cancer (BC) in juvenile age (mean, 20.2 yrs; range 19-21 yrs) and 19 BC patients (mean, 36.9 yrs; range 24-46 yrs) with a previous diagnosis of childhood/juvenile cancer (mean, 13.9 yrs; range, 1-20 yrs). All cases were negative for BRCA gene mutations and displayed no syndromic features. Patients' constitutional DNAs were submitted to whole exome sequencing on an Illumina HiSeq platform. After quality check, identified variants were selected using the Ingenuity Variant Analysis software. Only likely loss of function (LOF) variants (frameshift, stop gain, start loss and bioinformatically ascertained spliceogenic variants) with a read depth ≥40, an allelic fraction between 0.4 and 0.6 and a frequency <10⁻³ or nor reported, were considered. Overall, LOF variants in genes associated with high or moderate risk of BC, including ATM, BARD1, PALB2, POLB, POLD1 and RAD50, were observed in six cases (24%). Subsequently, based on the notion that all the above genes are, directly or indirectly, involved in DNA repair, we looked at genes whose activity mediates DNA metabolism and found two additional LOF variants in LIG4, coding for the DNA ligase IV enzyme, and ADA, coding for adenosine deaminase (the latter variant was detected in the same patients carrying the POLB variant). Our results provide direct support to the hypothesis that different BC predisposition and DNA repair genes may also be involved in the development of cancers at very early age. Larger genetic investigations and functional studies are necessary to validate this possibility. In addition, these studies might provide clues on the possible interactions between LOF variants in DNA repair genes and DNA damaging therapies for cancer treatment that might lead to the onset of secondary oncologic diseases.

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