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PS186

Epigenetic modifications as targets to new therapies for Chronic Lymphocytic leukaemia – A preliminary study

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Aim: This study aimed to clarify the involvement of epigenetic modifications in chronic lymphocytic leukaemia development and analyse the therapeutic potential of epigenetic modulators.

Introduction: CLL is the most common type of leukaemia found in adults and is an extremely variable and heterogeneous disease. The CLL aetiology is unknown and it natural history is heterogeneous. However, epigenetic modifications may play an important role in CLL.

Methods: This study enrolled 18 CLL and 7 controls. To perform primary CLL cultures, peripheral blood mononuclear cells from CLL patients were isolated using Ficoll gradient and incubated with the hypometilants, Azacytidine and Decitabine, and deacetylase inhibitors, Panobinostat and Vorinostat, in monotherapy (single dose and daily administration) and in combination for 24 h/48 h. The cytotoxic/cytostatic effect of drugs was evaluated by fluorometric microculture cytotoxicity assay (FMCA). Cell death and cell cycle were determined by flow cytometry using Annexin V and PI/RNAse, respectively. CD5 and CD19 antibodies were used to identify normal (CD5−/CD19+) and neoplastic cells (CD5+/CD19+). Methylation pattern was determined by MS-MLPA. Data were analysed using univariate approaches.

Results: Preliminary results show that patients appear to be more sensitive to Azacytidine and Vorinostat than Decitabine and Panobinostat, on single dose administration. Combination of Panobinostat with Azacytidine and Decitabine induced higher cytotoxicity than single dose. For all drugs, daily administration schedule reduced more effectively cell viability/proliferation than the same doses in single administration. These drugs induced cell death mainly by apoptosis with specificity to neoplastic cells. Moreover, CLL patients had a significant higher methylation frequency of PAX5 (70%), KLLN (80%), WT1 (100%), THBS1 (90%) and GATA5 (90%) gene promoters when compared with controls (all genes demethylated, except MSH6). Furthermore, all CLL patients had at least one methylated gene.

Conclusion: The preliminary results suggest that methylation of tumour suppressor genes is a common event in CLL patients and that epigenetic modulators induce a cytotoxic effect, reducing cell viability/proliferation, in a time- and dose-dependent manner. Therefore, these results are promising and encourage further studies in CLL.

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PS191

Imaging features of brain metastases from testicular cancer

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Aim: Our study evaluated the incidence, imaging characteristics, and prognosis of brain metastases originating from primary testicular tumors.

Introduction: Approximately 95% of testicular tumors are testicular germ cell tumors (TGCT).[1] Sertoli cell tumors are rare non-germ cell origin tumors and account for less than 1% of testicular cancer.[2] Brain metastases from germ cell tumors are very uncommon, occurring in less than 2–3% of patients.[3] In testicular cell cancer, it is estimated that the incidence of brain metastases is 1–2% in all TGCT, whereas in advanced stages of TGCT the incidence rises to about 10–15% [4–9]

Methods: Case records of testicular tumors patients within the IPO Porto data base from 2006 to 2015 were reviewed to identify patients with testicular tumors and evidence of brain metastases.

Results: 368 patients with testicular tumors were identified, with only four having evidence of brain metastases.

Histopathological evaluation reveled that one of the patients had a non-germ cell tumor, a Sertoli cell tumor, while others had mixed germ cell tumors. Half of them had only a single right frontoparietal lesion (21 mm) or right occipital (42 mm), both were heterogeneous in T1WI and T2WI, and with intense and heterogeneous enhancement with gadolinium. The other two patients had multiple lesions. One of them had left frontoparietal (2.2 mm, hyperintense in T1) and right occipital (1.8 mm, hypointense in T1) lesions, both heterogeneous and predominantly hypointense in T2 and T1WI with no enhancement. The other had right temporal (5 mm) and left occipital (11 mm) lesions, both isointense in T1W1 and T2WI with intense and homogeneous enhancement. There was no diffusion restriction in all three cases and all four cases were hypointense in T2*.

Conclusion: Although the imaging features of brain metastases differ in some aspects, they all have a hemorrhagic component and a very low survival rate after diagnosis.

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PS196

Synthesis and tumor cell growth inhibitory effects of the marine product analogues of fiscalin B

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Aim: The aim of this work was to synthesize fiscalin B, to pursuit the development of a library of derivatives and to investigate the derivatives for their potential antitumor activity.

Introduction: Marine organisms provided numerous novel compounds with sensational multiple pharmacological properties. The necessity of novel therapeutics has gain more importance especially because of the resistance associated to the current therapeutics and the inexistent treatments for incurable diseases. Fiscalin B is a fungal metabolite with a pyrazino[2,1-b]quinazoline-3,6-dione system reported to have significant biological activities.

Methods: Two methods were studied for synthesis – double cyclization and microwave assisted procedures. First method started with coupling reactions to form tripeptide of tryptophan methyl ester linked to N-Fmoc-valine via anthranilic acid. Then, the dehydrative cyclization was performed using formamide to form the intermediate oxazine. The coupling reaction to form the fiscalin B were achieved after deprotection.[1] The second method is the coupling of anthranilic acid with N-Boc-valine to form Boc-protected benzoxazin-4-one by thermal heating conditions. Then, the addition of tryptophan methyl ester hydrochloride led to 4-quinazoline-3,6-dione scaffold by microwave irradiation.[2] The cell growth inhibitory effect was investigated by the Sulforhodamine B assay.

Results: The use of amino acids with different configurations and different side chains or even the derivatization of the existing functional groups were enable the application of this synthetic methodology for a library of fiscalin B analogues. The formation yields of fiscalin B analogues were low, ranging from 3 to 16%. Eight derivatives were tested on non-small cell lung cancer (H460), colon adenocarcinoma (HCT15) and breast cancer (MCF7) human cell lines and showed moderate cytotoxic effects, with GI50 concentrations ranging from 30 to 80 μM.

Conclusion: Significant differences were obtained between enantiomeric pairs.

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