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Abstracts

MEDULLOBLASTOMA

MB-01. INVOLVEMENTS OF hsa-miR-383 AND ITS TARGET PEROXIREDOXIN 3 (PRDX3) IN CONTROLS OF MEDULLOBLASTOMA CELL GROWTH

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Medulloblastoma (MB) is the most common children brain cancer. Recent advances in cancer biology strMBongly suggest that impaired microRNAs expression is one of the critical events driving cancer development. Previous studies of microRNA expression profiling suggested hsa-miR-383 as one of the down-regulated microRNAs in MB. However, the functions of this microRNA in MB remain unclear. In this study, we demonstrated frequent down-regulation of hsa-miR-383 expression in MB by quantitative stem-loop-RT-PCR analysis. Twenty-three out of 29 (79%) MB samples expressed >2-fold of the lower level of hsa-miR-383 compared with normal cerebellar samples and also limited/non-detectable levels were found in 4 MB cell lines (DAOY, ONS-76, D283, and D458). Ectopic expression of hsa-miR-383 by microRNA mimic significantly inhibited MB cell growth along with increase of PARP cleavage, suggesting induction of apoptosis in the hsa-miR-383-mimic-treated MB cells and tumor suppressive roles of hsa-miR-383. By transcriptome analysis of hsa-miR-383-mimic-treated MB cells and computational prediction of hsa-miR-383 targets, we identified Peroxiredoxin 3 (PRDX3) as one of the targets with significant down-regulation of expression in the mimic-treated MB cells. Down-regulation was verified at both RNA and protein levels. In addition, the mimic significantly reduced luciferase activity of the reporter that was constructed with the 3'UTR of PRDX3 in MB cells. Site-directed mutation of the predicted recognition site abrogated the reduction, and this demonstrated the specificity of hsa-miR-383-mediated repression on PRDX3 expression in MB cells. Furthermore, siRNA knockdown of PRDX3 resulted in cell growth inhibition and induction of PARP cleavage, mimicking the effects of the hsa-miR-383 restoration by microRNA mimic in MB cells. In conclusion, hsa-miR-383 may function as tumor suppressive microRNA in MB and this is mediated through its target PRDX3 to control MB cell growth.

MB-02. QUESTIONABLE ROLE OF CRANIOSPINAL IRRADIATION (CSI) IN NON CEREBELLAR PNET(NCPNET) WHEN USING A HIGH-DOSE CHEMOTHERAPY (HDCT) STRATEGY

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BACKGROUND: A prospective intensive chemo-radiotherapy trial was launched for ncPNET. After introducing HDCT, we amended CSI to focal RT for selected cases. METHODS: From 1997 to 2010 we enrolled 25 consecutive patients in a pre-radiation schedule containing HDMTX, HDVP16, HDCTX and HDCBDCA, followed by HART-CSI at total doses 31-39 Gy, implemented with 2 hd thiotepa courses following CSI after the first 5 patients. Six children received the same chemotherapy but only focal RT at 54 Gy. RESULTS: 16 were males, median age was 7 years. Median follow-up was 42 mos (11-152). 5year PFS and OS were 56% and 47%, respectively, being PFS 80% for pineal tumors (n 10) vs 43% for other sites (p 0.05). Residual disease (n 22), metastases (4), response to pre-RT CT were not prognostically significant. Patients receiving HDCT had a PFS of 64% vs 37.5% (p 0.09). Response to RT in children with residual tumor was significant (3 year PFS 57% vs 42%, p 0.03) while it was not total CSI dose when used. Relapses were local in 6, local and disseminated in 2 and disseminated only in one case, not submitted to HDCT. Hence our decision to give only focal radiation to subsequent children providing they had no progression during pre-RT CT. Only 1/6 relapsed so far, locally (ETANTR diagnosis). CONCLUSIONS: This intensive schedule obtained a very satisfying outcome in pineoblastoma and allowed a further treatment stratification in radiation delivery, thus potentially saving a subgroup of children from full dose and CSI. Local relapse is in fact the main cause of treatment failure in ncPNET.

MB-03. MEDULLOBLASTOMA BELOW THE AGE OF 3 YEARS: TREATMENT AND PROGNOSTIC FACTORS

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BACKGROUND: Medulloblastoma patients below 3 years had inferior survival rates due to several reasons. AIM: To investigate the treatment end-results of medulloblastoma under 3 years of age and determine the factors that affects its prognosis. PATIENTS AND METHODS: Eighteen children below the age of 3 years were treated at Children's Cancer Hospital, Egypt during the period from July 2007 and December 2010. Safe maximum resections were attempted in all patients. Gross total resection was performed in 10 children (56%), subtotal excision in 7 children (39%) and biopsy in one patient. Fourteen children (78%) proved to be non-metastatic, while 4 belonged to M3 category (spinal seedling). Eight out of the 18 (44%) children received infantile medulloblastoma chemotherapy protocol, while the other 10 received other chemotherapy protocols. All the 4 metastatic children received craniospinal irradiation (CSI) with boost to the seedling site. Six out of the M0 patients received posterior fossa (PF) irradiation (5580 cgy), while the other 8 received CSI, as they reached the age of 3 years, with booster dose up to 5580 cGy to PF. RESULTS: The 3-year overall survival (OS) for all children was $55 \pm 16\%$. The OS for non-metastatic was $61 \pm 15\%$ and $50 \pm 29\%$ for M children. The infantile chemotherapy protocol led to 3-year OS of $71 \pm 17\%$ compared to $24 \pm 18\%$ for other protocols. The OS for CSI was $71 \pm 17\%$ compared to $49 \pm 25\%$ for conformal PF irradiation. None of the CSI group developed CNS relapse, while only one (17%) who received PF irradiation had spinal relapse. It is worthnoting that non of the these detected differences were statistically significant. All children tolerated treatment with minimal immediate toxicity and acceptable, so far, late effects. CONCLUSIONS: The 3-year OS of children below 3 years were modest with improved OS in non-metastatic patients who received infantile protocol and CSI.

MB-04. MEDULLOBLASTOMA HISTOLOGICAL VARIANTS AS THE MOST POWERFUL CLINICAL PROGNOSTIC INDICATOR: A 10-YEAR MONO-INSTITUTIONAL EXPERIENCE

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BACKGROUND: Histological classification in medulloblastoma has aroused in importance and newer treatment protocols will include histology as risk factor. We centrally revised all our medulloblastoma cases of the last ten years and re-assessed their histology to identify its prognostic importance. METHODS: Patients' samples were reviewed according to the two subsequent WHO classifications 2000 and 2007. Consecutive patients were 125. RESULTS: Male were 99, non-metastatic were 82, primary tumor was completely resected in 97, 10 patients were under 3-years of age. Median follow-up was 96 months. Treatment applied was institutional driven, hyperfractionated accelerated radiotherapy (HART) based, for 39 non metastatic cases before 2003, thereafter European PNET IV protocol was applied in 31, a HART based strategy was applied to 39 metastatic medulloblastoma children, a infants-tailored in 10 (high-dose-chemotherapy based), finally it was patient conditions tailored in 7. Five years PFS/EFS/OS were 76%, 73% and 81%, respectively. Histology was classic in 93 cases, desmoplastic in 20, anaplastic/large cell in 7/2 and with extensive nodularity (MBEN) in the last three. Prognostic stratification according to both presence of residual disease and metastases was not prognostic either, as were not age and the different protocols adopted. Histology gave 82% 5-year PFS for desmoplastic + MBEN variant, 78% for classic, and 44% for anaplastic/large cell one ($P = 0.009$). CONCLUSIONS: "Tailored" treatments to recognized risk factors have flattened prognostic differences, while histologic

revised anaplasia, that was not considered as such when designing previous trials, remained the most powerful prognostic factor and deserve appropriate treatment intensification.

MB-05. THE APPLICATION OF NANOPARTICLE LIPOSOME-IMPRAMINE BLUE IN THE TREATMENT OF MEDULLOBLASTOMA IN THE SmoA1 TRANSGENIC MICE

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Medulloblastoma is one of the most common malignant tumors in children. Its lethality is associated with tumor metastasis and the side effects of available treatment severely affect survivor's quality of life. Impramine blue (IB), a new anti-tumor drug, is encapsulated with liposome to form a liposomal nanoparticle (Liposome-IB), which has the advantage of reaching tumor via the enhanced permeability and retention effect. In vitro studies demonstrated that Liposome-IB inhibit the growth and migration of several medulloblastoma cells including Daoy (human) and Ps125 (mouse) in a dose-dependent manner. To determine the responsiveness of medulloblastom to liposome-IB in vivo, we carried out a preclinical survival study using the SmoA transgenic mouse model of medulloblastoma. Magnetic resonance imaging (MRI) was utilized for screening and detecting tumor in mice aged 12-15 week old. The tumor bearing mice were divided into treatment and control groups randomly. The mice in the treatment group received two doses of Liposome-IB by tail vein injection with a 5 day interval. The dosage of Liposome-IB is 4.5mg/kg (liposome-IB /body weight). The mice in the control group received the same amount of Liposome-only. The tumor progression was monitored by MRI at the different time points after the Liposome-IB treatment. The results showed that the tumor volume increased dramatically in the control group compared with Liposome-IB treatment group. The Liposome-IB treated mice survived significantly longer (median survivals of 82 ± 21.9 days) than the control mice (median survivals of 25 ± 15.5 days; $P = 0.024$). In conclusion, the nanoparticle Liposome-IB is effective in the treatment of mouse medulloblastoma in vivo. It can significantly delay the tumor progression and prolong the tumor bearing mice's survival time. The results provide valuable data in supporting the translation from the preclinical animal model trial to the development of clinical trial and protocol to the cancer patients.

MB-06. HOW TO REDUCE CHEMOTHERAPY FOR LOWER-RISK MEDULLOBLASTOMAS - IMPORTANCE OF INTERACTIONS BETWEEN SURGERY AND ADJUVANT THERAPY

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PURPOSE: ICE chemotherapy, comprising ifosfamide, cisplatin, and etoposide, is one of the most common regimens for medulloblastoma in Japan. However, hematological and hearing toxicities are frequently encountered. We report herein a single-institute experience of reduced chemotherapy for lower-risk medulloblastomas. METHODS: Thirteen patients with medulloblastoma treated at our institute from 2000 to 2011 were retrospectively reviewed. Adjuvant treatment was selected based on our new clinical classification. Tumors that have been completely resected, with no residual tumor apparent on MRI, in patients >3 years old with no leptomeningeal dissemination (M0) are classified as "lower risk". Patients with residual tumors $<1.5\ cm^2$ who are >3 years old and show no or only focal dissemination (M0-2) are classified as "average risk". Patients with residual tumors $\geq 1.5\ cm^2$, age <3 years or M3 are classified as "high risk". Seven patients were categorized to the lower-risk group, with 4 in the average-risk group and 2 in the high-risk group. Adjuvant treatment after surgery consists of radiotherapy, 24 Gy to the whole brain and spine plus 30 Gy to the posterior fossa, followed by chemotherapy. Chemotherapy for lower-risk patients consists of cisplatin and etoposide (CE chemotherapy), while ICE is used for average-risk ICE. RESULTS: Five-year survival and 5-year progression-free survival rates for the 11 patients of lower- and average-risk groups were 100% and 75%, respectively. Performance status scores for 10 of these 11 patients (91.0%) were 90-100%. Surgical complications, including postoperative intracranial hematoma and meningitis, were seen in recurrent cases and the low performance status cases. Introduction of radiation was delayed in these cases, with intervals between surgery and radiation of >40 days, compared to <28 days in the other 8 cases. CONCLUSIONS: For the lower-risk group, reduced chemotherapy was acceptable. Surgery with no complications that might delay adjuvant therapy is essential to reduce chemotherapy required.

MB-07. GORLIN'S SYNDROME (GS) AND DESMOPLASTIC MEDULLOBLASTOMA (DMB): REPORT OF 3 CASES WITH UNIQUE TUMOR LOCATION, CLINICAL COURSE, AND NOVEL MUTATION

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GS is a genetic condition associated with DMB in 5% of cases. DMB in GS usually occurs in infants and has a good prognosis. Radiotherapy (RT) is usually avoided in these children due to the higher risk of secondary cancers following RT exposure. We present three cases of GS with DMB with unique disease features. Patient #1 with frontal bossing, facial dysmorphism, bifid right 3rd rib, and a spontaneous germline *PTCH* mutation (C > T, exon 18) underwent gross total resection (GTR) of cerebellar DMB at age 2 years but focally relapsed in the right lateral ventricle following induction chemotherapy (IC). She underwent re-resection, IC, and high dose chemotherapy (HDC) + autologous stem cell rescue (ASCR), and no RT. She is 84+ months from HDC with no evidence of disease (NED). Patient #2 with mild frontal bossing and large hands and an inherited germline *PTCH* mutation (C > A, exon 12), underwent partial resection of a pineal tumor at age 20 months and found to be a DMB. He underwent IC followed by HDC + ASCR but had disseminated recurrence 6 months following treatment and subsequently died following palliative RT. Patient # 3 with frontal bossing, macrocephaly, synorphis, bifid right 3rd rib, and spontaneous germline mutation, c.1670 C > G, p. Thr557Arg), presented at age 2.5 years with a cerebellar DMB with excessive nodularity. She underwent GTR of tumor and IC followed by HDC + ASCR and no RT. She is now 10 + months following ASCR with NED. Although pts with GS and DMB have a good prognosis, disease progression can occur following initial therapy but can still be controlled using HDC + ASCR without RT. The association of GS with DMB in the pineal region or due to exon 12 1670 C > G, p. Thr557Arg mutation have not been previously reported.

MB-08. *MYC* AMPLIFICATION CAN BE SAFELY ASSESSED BY ARRAY-CGH IN MEDULLOBLASTOMAS

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As risk factors for poor outcome, *MYC* and *MYCN* amplifications are routinely assessed in medulloblastomas. FISH is considered as the technique of reference. Recently, array-CGH has been developed as an alternative technique to evaluate genomic abnormalities in many other tumour types. However, this technique has not been validated in medulloblastomas. MATERIAL AND METHODS: we systematically compared FISH and array-CGH on a retrospective series of medulloblastomas referred to our institution from 2007 to 2011. FISH were performed on frozen section, using *MYC* and *MYCN* probes (Vysis, Abbott Molecular, Des Plaines, IL). Array-CGH were done on home-made BAC-arrays until 2009, and then on 4*72K arrays NimbelGen (Madison, WI). Before DNA extraction, the tumour cellularity was evaluated on a matched frozen section; all tumours showed >80% tumour cells. RESULTS: we screened 72 tumours by FISH; *MYC* and *MYCN* amplifications were evidenced in 4 and 7 cases, respectively. In all cases showing amplification by FISH, array-CGH unambiguously revealed the abnormality. Array-CGH was also done on 25 tumours showing no amplification by FISH; consistently, no amplification was detected. Interestingly, one tumour showed a focal *MYC* amplification by FISH, observed in approximately 20% of tumour cells; this subclonal amplification was clearly evidenced on array-CGH. CONCLUSION: our analysis confirms that array-CGH is as safe as FISH for the detection of *MYC* genes amplification. Even in case of focal amplification, a theoretical event which is very rare in routine practice, array-CGH is sensitive enough to detect the subclonal abnormality. Since the percentage of tumour cells reaches at least 80% in virtually all medulloblastomas, the issue of contamination by normal cells is marginal. Given their cost effective price in comparison to two FISH tests and the wide genomic information provided by array-CGHs, this reproducible technique can be safely retained as an alternative to FISH for daily routine medulloblastomas investigation.