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Abnormal expression of P2X family receptors in Chinese pediatric acute leukemias

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ABSTRACT

Nucleotides are new players in intercellular communication network. P2X family receptors are ATP-gated plasma membrane ion channels with diverse biological functions. Their distribution patterns and significance in pediatric leukemias have not been established. Here we investigated the expression of P2X receptors in BMMC samples from Chinese pediatric acute leukemias. Real-time PCR and Western blot results showed that P2X1, P2X4, P2X5 and P2X7 receptors were simultaneously over expressed in leukemias compared with controls, whereas P2X2, P2X3 and P2X6 were absent or marginally expressed in both groups. It was worth noting that the co-expression feature of them, especially between P2X4 and P2X7, could be observed and the highest expression of P2X7 was detected in relapsed patients. Moreover, concomitant decrease of P2X4, P2X5 and P2X7 expressions was observed at CR stage in a follow-up study. Functional P2X7 was also verified. These results suggested that P2X1, P2X4, P2X5 and P2X7 were hematopoiesis-related P2X receptors, and their signaling, especially for P2X7, might play important roles in pediatric leukemias. P2X receptors might co-operatively contribute to the malignant phenotype in human pediatric leukemias.

Introduction

The abnormal signaling from the microenvironment of malignancies contributes to the malignant phenotype of tumor cells. Besides abnormal existence of peptide molecules, abnormal level of extracellular nucleotides, such as ATP, could be observed in tumor microenvironment [1]. Nucleotides, as a ubiquitous family of extracellular signaling molecules, exert different effects through the interaction with P2 receptors, which are classified into P2Y and P2X families. P2Y family receptors belong to seven-membrane-spanning G protein-coupled receptors [2], while the P2X receptors including seven distinct subtypes (P2X1–P2X7) are ATP-gated plasma membrane ion channels that mediate transmembrane cation fluxes [3].

The P2X family receptors are widely distributed with various functions, which suggest that they may participate in both physiological and pathological processes [4]. In fact, accumulating evidence demonstrated that P2X receptors were involved in pathophysiological processes including inflammation [5], and several cancers [6,7]. It was recently indicated that co-expression of different P2X receptor subtypes might influence the function of a single submit [8], which suggested that it's necessary to know the expression of all seven subtypes of this family on any particular cell type or in any particular process for the better understanding the roles of these receptors.

In hematopoietic system, studies were focused on P2X7 expression and functions, though the expression of some P2X was reported in blood cells, and $CD34^+$ stem/progenitor cells [9–11]. Upon stimulation by extracellular ATP, a series of effects on cell proliferation, differentiation, chemotaxis, cytokine release, were observed [12]. Furthermore, the expression and function of P2X family receptors (mainly P2X7) were also studied in hematological malignancies. The high level expression of P2X7 was reported to be related to the disease severity of B-cell chronic lymphocytic leukemia (CLL) [13]. Our previous work demonstrated that high level expression of P2X7 was observed in leukemias and myelodysplastic syndromes (MDS) [14]. Dysfunction of P2X7 was observed in leukemia cell lines [14,15]. However, the full expression spectrum and significance of P2X family receptors in hematopoietic malignancies have not been established.

Childhood acute leukemias (AL) show unique clinical and biological features. Besides the frequent subtypes, the overall outcome of childhood leukemias is generally favorable, with event-free

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survival rates exceeding 80% in ALL [16], and over 50% in AML [17]. However, there has been no complete and systemic study on P2X family receptors in childhood neoplastic hematologic disorders. Our results demonstrated the simultaneous high expression of particular P2X receptors in human childhood leukemias. Moreover, significant decrease of their expressions was observed at complete remission stage after chemotherapy.

Materials and methods

Patients, and sample preparation. Bone marrow (BM) samples were obtained from 124 patients with diagnosed childhood AL who were younger than 18 years at initial diagnosis, including 66 cases were newly diagnosed ALL (11 T-ALL and 55 B-ALL), 37 cases were newly diagnosed AML, 21 cases were relapsed patients (17 ALL and 4 AML). In 26 patients (17 newly diagnosed ALL, 3 relapsed ALL and 6 newly diagnosed AML), bone marrow samples were obtained at both before and after standard chemotherapy reaching complete remission (CR). A total of 20 childhood donors were obtained as the control group. In all cases, written informed consent was given and was approved by our institutional review board.

Bone marrow mononuclear cells (BMMCs) were prepared by Ficoll-Hypaque densitygradient centrifugation.

cDNA synthesis and relative quantitative real-time PCR. Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions. cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen) following the standard protocol.

The mRNA expression levels of P2X family receptors and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured by relative quantitative real-time PCR using an ABI 7500 Sequence Detector System (Applied Biosystems, Foster City, CA). The PCR amplification was performed using the SYBR Premix Ex Taq kit (TaKaRa Biotech). All reactions were performed in duplicate. The primers used for real-time PCR were listed (Supplementary Table 1). Analysis was performed using ABI 7500 Sequence Detection software (Applied Biosystems). The expression levels of the P2X receptors were analyzed by the RQ value calculated through ΔΔCt method $[\Delta\Delta Ct = (Ct_{P2X} - Ct_{GAPDH})_{sample} - (Ct_{P2X} - Ct_{GAPDH})_{calibrator}]$. The average ΔCt ($Ct_{P2X} - Ct_{GAPDH}$) from 20 controls combined was defined as calibrator, and the RQ of calibrator was 1.000. The amplification efficiencies for P2X and GAPDH were validated showing the same slopes. A negative control without template was included. Human leukemia cell line J6-1 [18] was used as a positive control. The PCR products of P2X(1–7) receptors and GAPDH from J6-1 were verified by DNA sequencing.

Western blotting. Cell lysis buffer was purchased from Cell Signaling Technology. As primary antibody, polyclonal antibodies against P2X1, P2X4, P2X5 receptors (Santa Cruz Biotechnology, Inc.) were used at 1:200, anti β-actin (Santa Cruz Biotechnology) and against P2X7 receptor (Sigma–Aldrich, Zwijndrecht, The Netherlands) were used at 1:1000. Anti–rabbit IgG horseradish peroxidase-conjugated antibodies at 1:5000 was then used as the secondary antibody. Visualization of specific proteins was carried out by an enhanced chemiluminescence method using ECL Western blotting detection reagents (Pierce Biotechnology, Rockford, IL, USA).

Measurements of intracellular free Ca^{2+}. Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined using the fluorescent indicator fura-2/AM [14]. Briefly, cells were incubated with 3 μM fura-2/AM (Sigma) at 37 °C for 20 min before washed twice with Locke's solution and resuspended. The cell suspension was then placed in the chamber of a dual excitation beam spectrophotometer (F-4500, Hitachi, Tokyo) with continuous stirring by a stirring bar. The fluorescence intensities at 510 nm were recorded when excited at 340 and 380 nm, simultaneously. BzATP (Sigma) was added to a final concentration of 100 μM. Triton X-100 was added to obtain maximal fluorescence and then excess EDTA was added to obtain minimal fluorescence. $[Ca^{2+}]_i$ was calculated using F4500 Intracellular Cation Measurement System (Version 1.02) software. In blocking experiment, KN62 (Sigma) was pre-incubated for half an hour before analysis.

Statistical analysis. After logarithm transformation, the RQ values of all the samples were shown as a normal distribution, so parametric test was used in all the statistical analysis. Two-tailed Student's *t*-test was used to test for differences between two groups; Kruskal–Wallis test (ANOVA) was used to test for differences among more than two independent groups. The pearson correlation and linear regression test were used for the associations between P2X receptors expression levels and some clinical indexes. Analysis was done using the SPSS software package (SPSS, Chicago, IL, USA). Statistical significance was accepted when the *P* values were less than 0.05.

Results

Expression analysis of P2X7 receptor in childhood acute leukemias

The expression of P2X7 was analyzed in 124 patients. The expression level of P2X7 was significantly higher in newly diagnosed ALL ($p = 0.020$) or AML ($p = 0.018$) patients than in normal controls. Furthermore, it was much higher in ALL patients with relapse than either newly diagnosed patients ($p = 0.033$) or the normal control ones ($p = 0.000$), whereas no difference was found in AML with relapse group due to the limited cases ($n = 4$). No significant difference could be detected on its expression between the ALL and AML groups, or between B-ALL and T-ALL groups (Fig. 1A).

The results from the follow-up study showed that its expression at CR stage decreased dramatically when compared with that before chemotherapy. Statistical difference could be detected in both AL group ($n = 26$, $p = 0.00$) and ALL group ($n = 20$, $p = 0.00$) (Fig. 2A).

Statistic analysis of ALL samples was also performed on sex, age, leukocyte count, LDH level, numerical or structural cytogenetic abnormalities, 7 day peripheral blood response to prednisone treatment, risk group, and no statistic significance was found (Supplementary Table 2).

The expression of P2X7 was also detected in parallel in 12 ALL and 9 healthy control cases by Western blotting. We demonstrated that the P2X7 protein was significantly higher in patient samples than that in control samples (Fig. 4A), which is in accordance with the mRNA level results.

Expression of P2X(1–6) receptors in childhood acute leukemias

The expression of P2X1 was analyzed in 115 cases. Its expression was significantly higher in newly diagnosed ALL ($p = 0.04$) and AML ($p = 0.00$) patients, especially in ALL/AML ($p = 0.02/0.04$) patients with relapse, than in normal controls. Though no significant difference could be detected between ALL and AML, newly diagnosed ALL and the relapsed ALL, or among AML different sub-groups, B-ALL patients ($n = 48$) showed significantly higher P2X1 expression ($p = 0.000$) than that of T-ALL cases ($n = 10$) (Fig. 1B).

The expression level of P2X4 from 124 cases revealed that it was significantly higher in newly diagnosed ALL ($p = 0.000$) and AML ($p = 0.000$) patients than in normal controls. It's much higher in ALL patients with relapse than the normal control group ($p = 0.001$), whereas no significant difference could be detected between newly diagnosed ALL and the relapsed ALL, between the ALL and AML groups, (Fig. 1C).

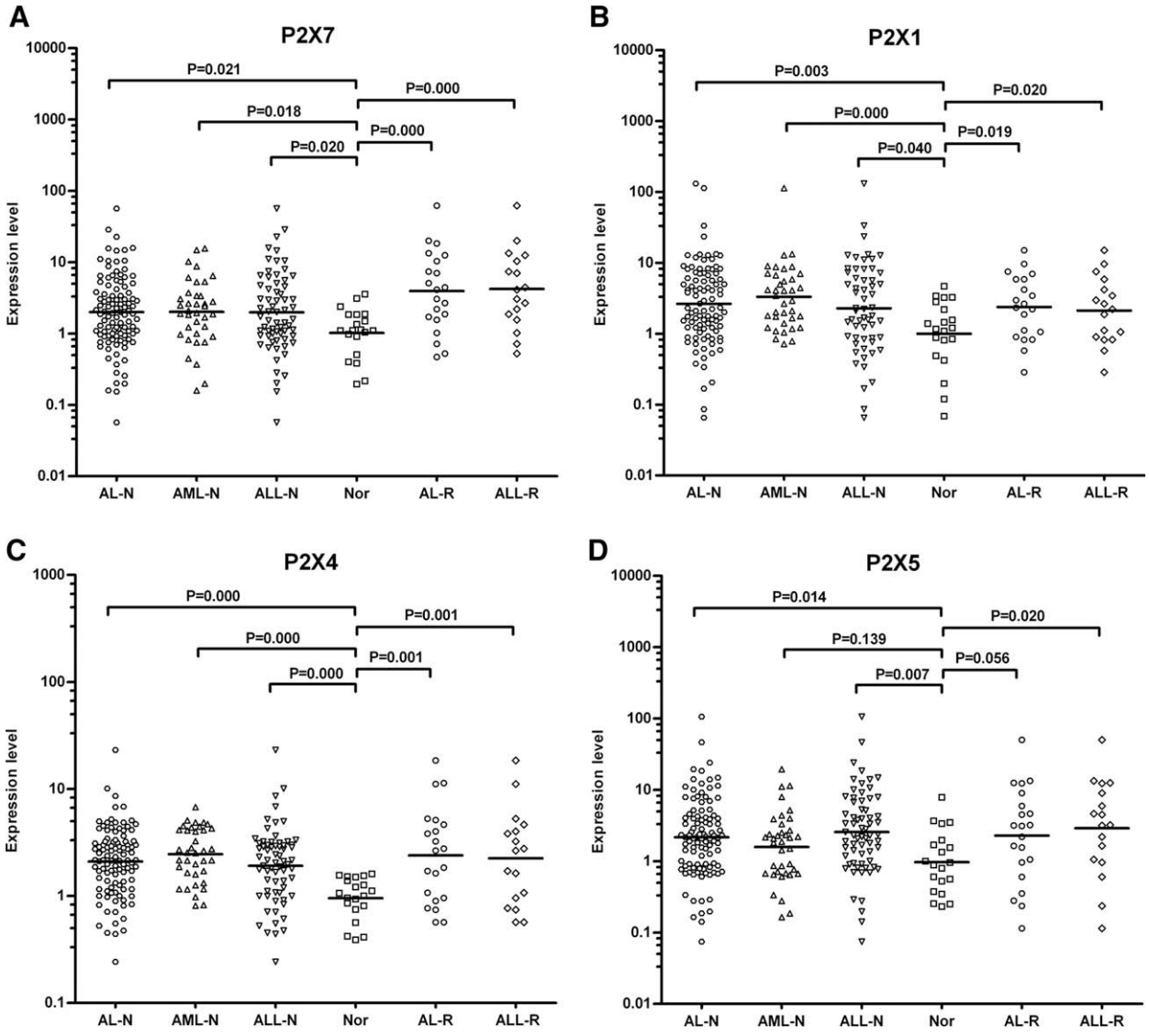

Fig. 1. Expression of P2X1, P2X4, P2X5 and P2X7 receptors in pediatric acute leukemia patients. BMMCs were isolated from 20 normal controls (Nor) and 124 childhood acute leukemia (AL) patients. The cases were further classified into newly diagnosed acute leukemia (AL-N), AL with relapse (AL-R), newly diagnosed acute myeloid leukemia (AML-N), newly diagnosed acute lymphoblastic leukemia (ALL-N), and ALL with relapse (ALL-R) sub-groups. The expression of P2X receptors was determined by real-time PCR detailed in Materials and methods. The expression level was assessed as the RQ value, and shown on the logarithmic *Y*-axis. The median RQ value of each group was also shown.

The data from 124 cases demonstrated that the abnormally high expression of P2X5 could only be detected in newly diagnosed ALL ($p = 0.007$) patients, but not in AML patients, when compared with normal controls. No significant difference could be detected between the ALL and AML groups, between relapsed patients and newly diagnosed ones (Fig. 1D).

In the follow-up studies, the expression of P2X4 and P2X5 receptors decreased dramatically at CR stage. Statistical differences could be detected in both AML ($n = 6$; $p = 0.015$ for P2X4 and $p = 0.02$ for P2X5) and ALL groups ($n = 20$; $p = 0.000$ for P2X4 and $p = 0.04$ for P2X5) (Fig. 2C and D). However, no statistical significant decrease could be found at CR stage for P2X1 receptor, though its expression in either ALL or AML was lower when compared with that before chemotherapy (Fig. 2B).

Statistic analysis of ALL samples was also performed on clinical features listed above. The results showed that P2X1 was highly expressed in TEL/AML positive B-ALL patients than in negative ones ($p = 0.017$). Interestingly, the expression of P2X1 was negatively related to age in ALL patients ($r = -0.44$; $p = 0.001$), but not in AML or normal controls (Supplementary Table 2).

The protein expression levels of P2X1, P2X4 and P2X5 were also detected in parallel in 12 ALL and 9 healthy control cases by Western blotting, demonstrating the higher expression of P2X1, P2X4 and P2X5 receptors in ALL patients than healthy controls (Fig. 4A), which is in accordance with the mRNA level results.

Undetectable or very low expression of P2X2, P2X3 or P2X6 receptor was detected in leukemia and control samples. This result was in accordance with our previous studies on leukemia cell lines.

The co-expression pattern of P2X family receptors in AL patients

As P2X1, P2X4, P2X5 and P2X7 receptors were highly expressed in childhood leukemia patients, it's interesting to know whether there is any expression correlation. High correlation between P2X4 and P2X7 receptors could be observed in AL patients ($r = 0.63$; $p < 0.0001$), but not in normal controls ($r = 0.33$; $p = 0.15$). (Fig. 3A and B). Middle levels of correlation were shown in both P2X5/P2X7 ($r = 0.41$, $p < 0.0001$) and P2X4/P2X5 ($r = 0.41$, $p < 0.0001$) pairs in AL (Fig. 3C and E), whereas it was higher in

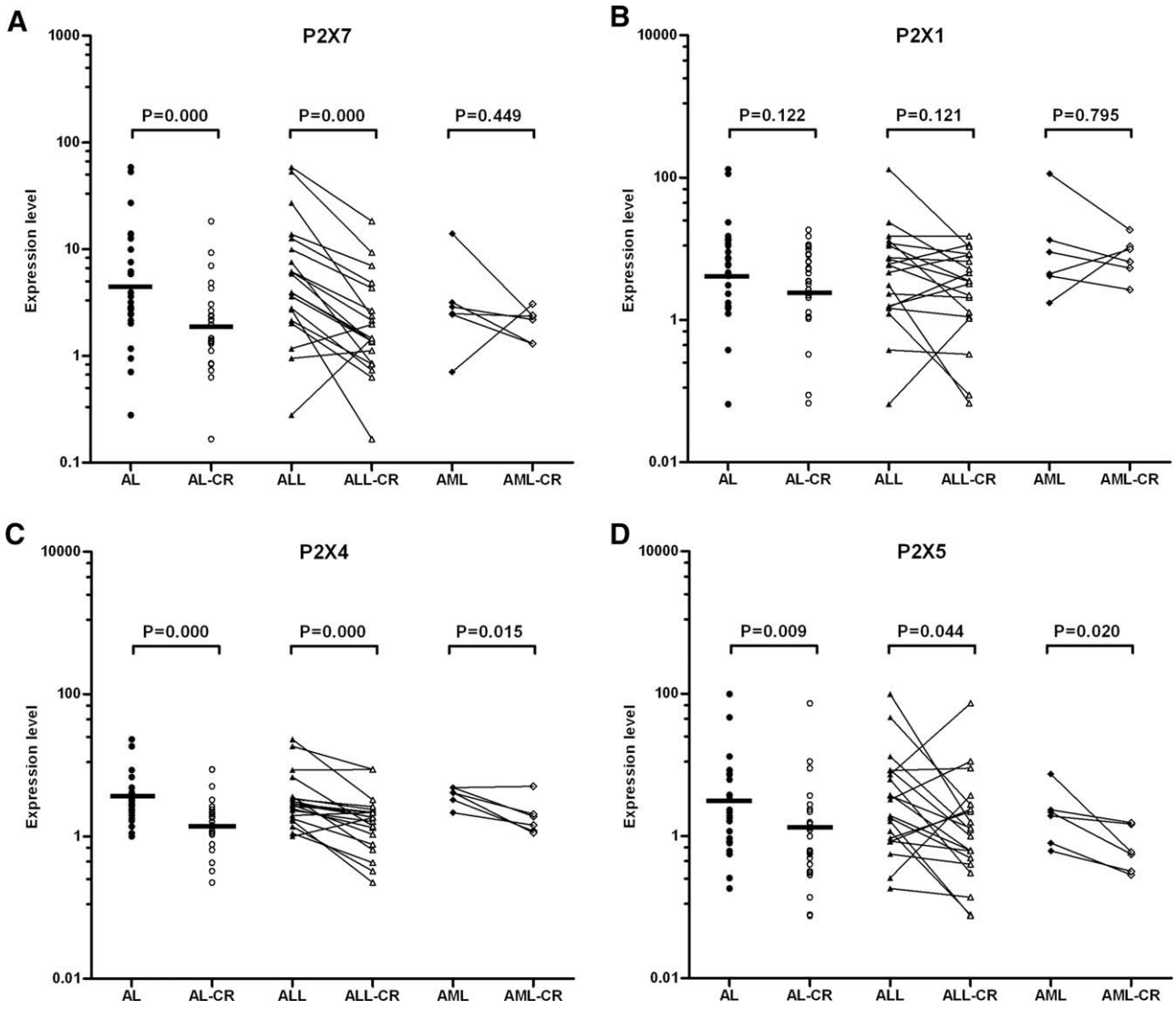

Fig. 2. Expression of P2X1, P2X4, P2X5 and P2X7 receptors in a follow-up study. BMMCs were obtained from 26 AL patients before and after standard chemotherapy reaching complete remission (CR). The expression of P2X receptors was determined by real-time PCR detailed in Materials and methods. The expression level was assessed as the RQ value, and shown on the logarithmic *Y*-axis. AL, ALL, AML: samples from AL, ALL or AML patients before chemotherapy; AL-CR, ALL-CR, AML-CR: samples from AL, ALL or AML patients at CR stage after chemotherapy. Lines connecting individual points from the same patients were shown in ALL and AML groups and the median of RQ values in AL groups were shown.

normal controls ($r = 0.79$, $p < 0.0001$ for P2X5 and P2X7; $r = 0.48$, $p = 0.03$ for P2X4 and P2X5) (Fig. 3D and F).

The expressions of other pairs of receptors were also studied. Weak correlation in either P2X1/P2X7 or P2X1/P2X4 pairs could be observed in AL patients ($r = 0.31$, $p = 0.002$ for P2X1 vs. P2X7; $r = 0.36$, $p = 0.0004$ for P2X1 vs. P2X4). However, higher correlation between P2X1 and P2X7 ($r = 0.58$; $p = 0.008$), but not between P2X1 and P2X4 ($r = 0.26$; $p = 0.273$) was found in normal controls.

BMMCs from patients and controls express functional P2X7 receptor

As we have shown the higher expression of P2X7 in newly diagnosed and relapsed childhood ALL, it's interesting to know whether the receptor was functional. As BzATP was the specific, complete and most potent agonist for P2X7 and could cause increase $[Ca^{2+}]_i$, we tested whether BzATP could induce $[Ca^{2+}]_i$ increase in some ALL and control samples. Fig. 4B and C showed $[Ca^{2+}]_i$ increase in BMMCs from ALL patients and healthy controls when stimulated with 100 μM BzATP. However, the $[Ca^{2+}]_i$ increasing tendency of ALL BMMCs was different from that of healthy control BMMCs. The $[Ca^{2+}]_i$ increase could be blocked by KN62, the most specific antagonist of P2X7, which suggested the calcium response was P2X7 receptor specific (Fig. 4D and E).

Discussion

Intercellular communication is the most important aspect modulating cell functions in multi-cell organisms. Abnormal communication leads to dysfunction of cells and various kinds of diseases. For decades, peptide signal molecule mediated intercellular communication has been well established. Nucleotides are new players in this field [19]. Their effects and significance in physiological and pathological processes are largely unknown. Hence, the knowledge of their expressions under disease conditions will help us for the full understanding of the particular disease and finding of potential therapeutic targets.

The expression of P2X receptors has been reported in a variety of tissues and cells, whereas their expression spectrum seems to be cell type specific [20]. Here, we demonstrate that four kinds of P2X

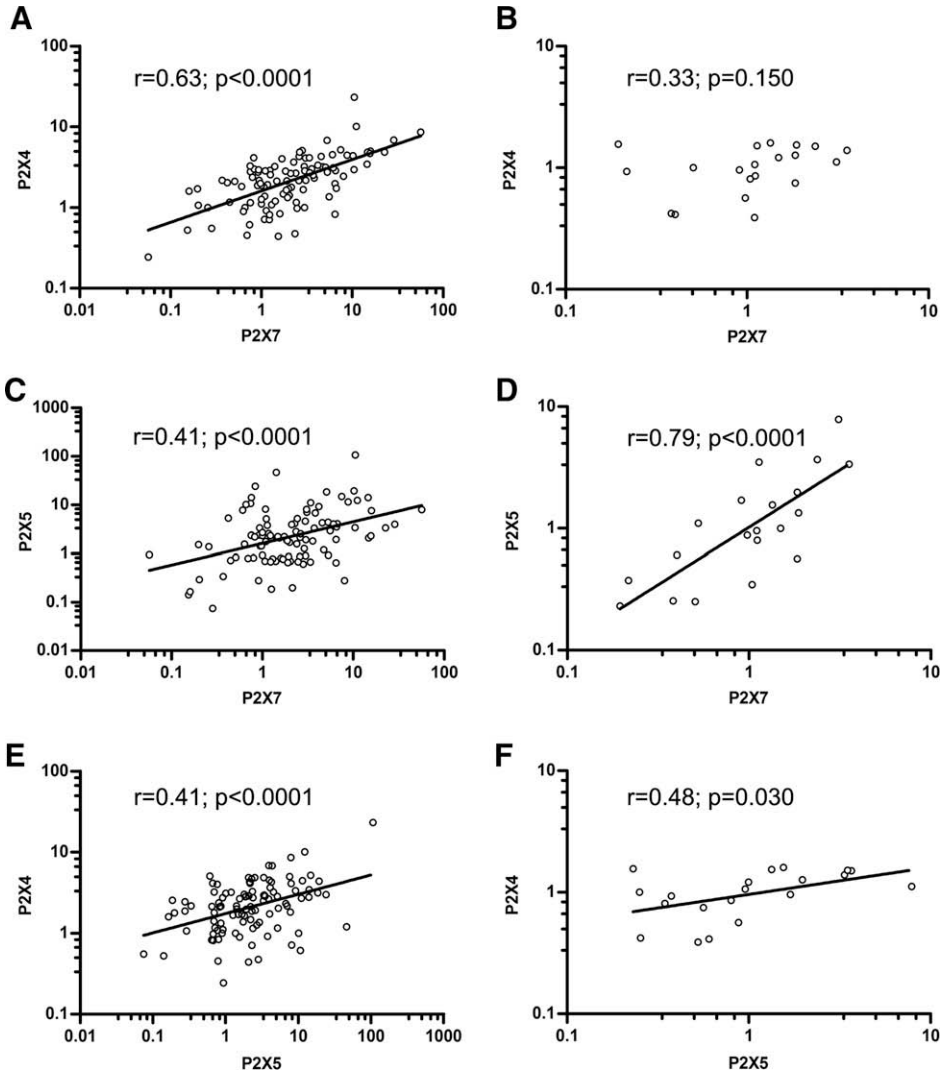

Fig. 3. The co-expression properties of P2X receptors in pediatric acute leukemias. BMMCs were isolated from 20 normal controls and 103 newly diagnosed childhood acute leukemia patients. The expression of P2X receptors was determined by real-time PCR detailed in Materials and methods. The expression level was assessed as the RQ value, and shown on the logarithmic axis. The co-expression of P2X receptors was analyzed in normal controls (B, D, F) and newly diagnosed childhood acute leukemia patients (A, C, E) using Pearson correlation and linear regression by SPSS. Regressive lines were indicated when the *r* value was greater than 0.4.

receptors (P2X1, P2X4, P2X5 and P2X7) are simultaneously expressed in human hematopoietic cells from both childhood normal and acute leukemia samples, whereas three receptors (P2X2, P2X3 and P2X6), which are mainly distributed throughout the nervous system, were absent or expressed at very low level, and this result was in accordance with our preliminary work in human leukemia cell lines. Our results suggested that P2X receptors might be subdivided into two sub-groups according their expression in hematopoiesis system, i.e. the hematopoiesis-related P2X receptors and hematopoiesis-unrelated P2X receptors.

An interesting finding of this paper is the simultaneous high expression of the hematopoiesis-related P2X receptors in childhood leukemias. Moreover, the co-expression feature of them, especially between P2X4 and P2X7 receptors could be observed. P2X family receptors shared homology and could form homo or hetero trimer upon stimulation. The channel co-assembled by various P2X subunits showed distinct functional properties from that assembled by either subunit [21,22]. Recently, a functional P2X4/P2X7 heteromeric receptor had been reported demonstrating a structural and functional interaction between P2X4 and P2X7 subunits [23]. Hence, it raised the speculation that the simultaneously high expression of hematopoiesis-related P2X might change the functional properties and might have particular roles in childhood leukemias, which need further verification. Our results suggested that the monitor of hematopoiesis-related P2X receptors may be more useful rather than a single subtype, such as P2X7.

The encouraging finding of our work is the concomitant decrease of the hematopoiesis-related P2X receptors at CR stage in a follow-up study. Nevertheless, they showed distinct feature in human childhood leukemias. Dramatic decrease could be observed for P2X4, P2X5 and P2X7, whereas slight decrease could be detected for P2X1, which suggested that those three receptors were closely related to the stage of disease and could become potential clinical markers monitoring chemotherapy. Though their roles in childhood leukemias were needed to be established, it had been proposed that P2X7 receptor over-expressing cells had the proliferative advantage [24]. Hence, over-expression of these three receptors might provide proliferative advantage in childhood leukemias. However, in relapsed leukemia group, much higher expression could only be found for P2X7 which suggested that over-expression of P2X7, but not P2X4 or P2X5, was related to relapse in childhood leukemias.

The expression pattern and significance of P2X7 in childhood leukemias had not been elucidated, though its high level expression

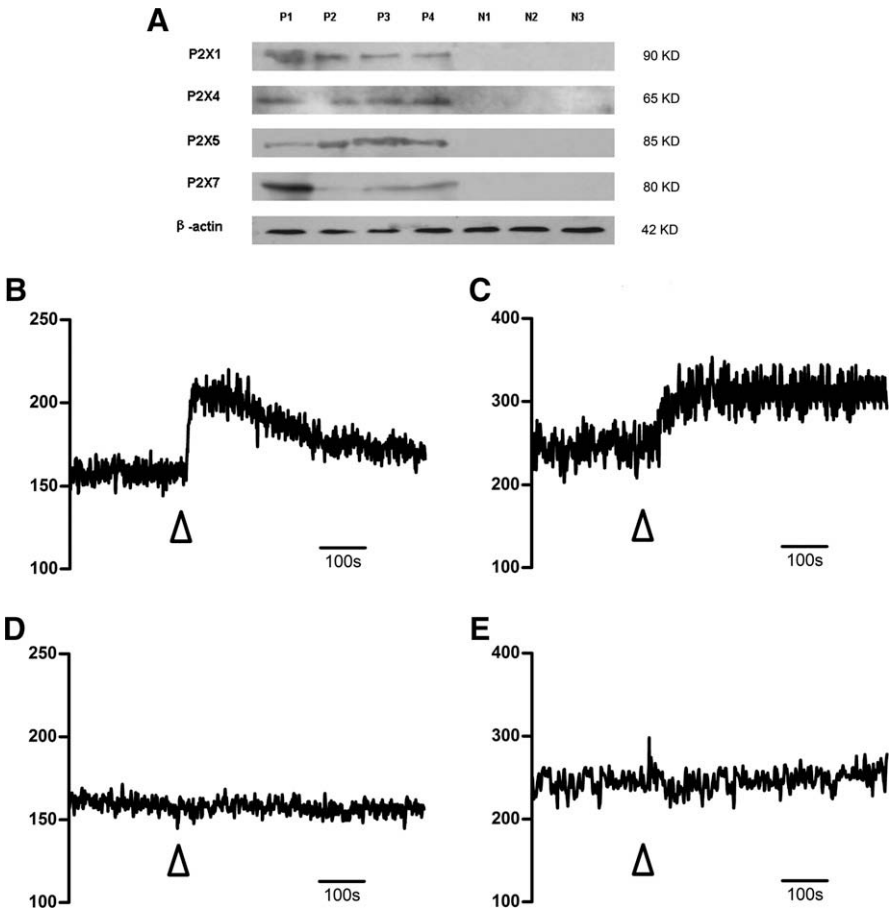

Fig. 4. Expression of P2X1, P2X4, P2X5 and P2X7 receptors and functional analysis of P2X7 receptor in pediatric acute leukemia samples. (A) The expression of P2X1, P2X4, P2X5 and P2X7 receptors at protein levels in 12 ALL and 9 control samples were studied by means of Western blotting using specific antibodies against the receptors and a control antibody against β-actin detailed in Materials and methods. A typical result is shown including four ALL (P1–4) and three normal control (N1–3) samples. (B–E) P2X7 receptor function in ALL and control samples was studied by detecting cytoplasmic free calcium $[Ca^{2+}]_i$ upon specific agonist BzATP using the fluorescent indicator fura-2/AM by spectrophotometer (F-4500, Hitachi). BzATP (100 μM) was added at the time point indicated (Δ). $[Ca^{2+}]_i$ is shown on *Y*-axis in nM. The time scale of 100 s was indicated. The figures represented the typical results from at least three independent experiments.

was reported in B-CLL [13] as well as human AL and MDS, which was mainly from adult samples [14]. We reported here the high expression of P2X7 receptor in newly diagnosed childhood leukemias and much higher levels in cases with relapse, while significant decrease could be observed after chemotherapy. These results strongly suggested that P2X7 receptor-mediated signaling might play important roles in childhood leukemias. In fact, P2X7 receptor was first regarded as a cytotoxic receptor due to its ability to form cytolytic pore leading to necrosis or apoptosis [25]. However, increasing data had implicated P2X7 as a survival/growth-promoting receptor, related to cell proliferation and tumor transformation [26–28]. ATP could be released under physiological conditions or as a consequence of cell damage, bacterial infection, or other noxious stimulus. In tumor environment, sustained high level of ATP was detected [1], which was in principle sufficient to activate P2X7 receptor. The signaling events downstream P2X7 receptor activation had been extensively studied, demonstrating the activation of ERK/JNK/p38 cascades [29–31] and phospholipase D [32], suggesting the complexity of its function.

Though the molecular mechanisms need to be elucidated, especially for P2X1, P2X4 and P2X5 receptors, our results suggested that they might play important roles in childhood leukemias. So far, it is also interesting to further elucidate whether the abnormal expressions of these hematopoiesis-related P2X receptors are related to the prognosis, survival of patients. The knowledge will help us for better understanding of intercellular communication in leukemias and exploring the potential target for the treatment of leukemias.

Contributions

J.-H. Chong provided the concept and design, contributed to the data analysis and interpretation, drafting of the article, collected and assembled the data, and gave final approval. G.-G. Zheng contributed to the concept and design, the data analysis and interpretation, provided critical revisions, gave final approval and obtained funding. X.-F. Zhu, Y. Guo, L. Wang, C.-H. Ma, S.-Y. Liu, L.-L. Xu, Y.-M. Lin, K.-F. Wu contributed to data analysis and interpretation, provided critical revisions and gave final approval.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.087.

References

[1] P. Pellegatti, L. Raffaghello, G. Bianchi, F. Piccardi, V. Pistoia, F. Di Virgilio, Increased level of extracellular ATP at tumor sites: in vivo imaging with plasma membrane luciferase, PLoS One 3 (2008) e2599.

[2] E.A. Barnard, G. Burnstock, T.E. Webb, G protein-coupled receptors for ATP and other nucleotides: a new receptor family, Trends Pharmacol. Sci. 15 (1994) 67–70.

[3] S. Valera, N. Hussy, R.J. Evans, N. Adami, R.A. North, A. Surprenant, G. Buell, A new class of ligand-gated ion channel defined by P2X receptor for extracellular ATP, Nature 371 (1994) 516–519.

[4] B.S. Khakh, R.A. North, P2X receptors as cell-surface ATP sensors in health and disease, Nature 442 (2006) 527–532.

[5] J.M. Labasi, N. Petrushova, C. Donovan, S. McCurdy, P. Lira, M.M. Payette, et al., Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response, J. Immunol. 168 (2002) 6436–6445.

[6] N. White, G. Burnstock, P2 receptors and cancer, Trends Pharmacol. Sci. 27 (2006) 211–217.

[7] A. Solini, S. Cuccato, D. Ferrari, E. Santini, S. Gulinelli, M.G. Callegari, et al., Increased P2X7 receptor expression and function in thyroid papillary cancer: a new potential marker of the disease?, Endocrinology 149 (2008) 389–396

[8] L. Koles, Z. Gerevich, J.F. Oliveira, Z.S. Zadori, K. Wirkner, P. Illes, Interaction of P2 purinergic receptors with cellular macromolecules, Naunyn Schmiedebergs Arch Pharmacol. 377 (2008) 1–33.

[9] M.P. Mahaut-Smith, G. Tolhurst, R.J. Evans, Emerging roles for P2X1 receptors in platelet activation, Platelets 15 (2004) 131–144.

[10] S. Berchtold, A.L. Ogilvie, C. Bogdan, P. Muhl-Zurbes, A. Ogilvie, G. Schuler, A. Steinkasserer, Human monocyte derived dendritic cells express functional P2X and P2Y receptors as well as ecto-nucleotidases, FEBS Lett. 458 (1999) 424–428.

[11] L. Wang, S.E. Jacobsen, A. Bengtsson, D. Erlinge, P2 receptor mRNA expression profiles in human lymphocytes, monocytes and CD34+ stem and progenitor cells, BMC Immunol. 5 (2004) 16.

[12] F. Di Virgilio, P. Chiozzi, D. Ferrari, S. Falzoni, J.M. Sanz, A. Morelli, et al., Nucleotide receptors: an emerging family of regulatory molecules in blood cells, Blood 97 (2001) 587–600.

[13] E. Adinolfi, L. Melchiorri, S. Falzoni, P. Chiozzi, A. Morelli, A. Tieghi, et al., P2X7 receptor expression in evolutive and indolent forms of chronic B lymphocytic leukemia, Blood 99 (2002) 706–708.

[14] X.J. Zhang, G.G. Zheng, X.T. Ma, Y.H. Yang, G. Li, Q. Rao, et al., Expression of P2X7 in human hematopoietic cell lines and leukemia patients, Leuk. Res. 28 (2004) 1313–1322.

[15] K. Nie, G.G. Zheng, X.J. Zhang, Y.M. Lin, L. Wang, G. Li, et al., CD39-associated high ATPase activity contribute to the loss of P2X7-mediated calcium response in LCL cells, Leuk. Res. 29 (2005) 1325–1333.

[16] C.H. Pui, J.T. Sandlund, D. Pei, D. Campana, et al., Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital, Blood 104 (2004) 2690–2696.

[17] G.J. Kaspers, C.M. Zwaan, Pediatric acute myeloid leukemia: towards high-quality cure of all patients, Haematologica 92 (2007) 1519–1532.

[18] K.F. Wu, Q. Rao, G.G. Zheng, Y.Q. Geng, M. Li, J. Kong, et al., Enhancement of J6-1 human leukemic cell proliferation by cell–cell contact: role of an M-CSF-like membrane-associated growth factor MAF-J6-1, Leuk. Res. 18 (1994) 843–849.

[19] G. Burnstock, Purinergic signalling: past, present and future, Braz. J. Med. Biol. Res. 42 (2009) 3–8.

[20] G.H. Bourne, One hundred volumes of the international review of cytology, Int. Rev. Cytol. 100 (1987) 1–13.

[21] A. Nicke, D. Kerschensteiner, F. Soto, Biochemical and functional evidence for heteromeric assembly of P2X1 and P2X4 subunits, J. Neurochem. 92 (2005) 925–933.

[22] U. Lalo, Y. Pankratov, S.P. Wichert, M.J. Rossner, R.A. North, F. Kirchhoff, A. Verkhratsky, P2X1 and P2X5 subunits form the functional P2X receptor in mouse cortical astrocytes, J. Neurosci. 28 (2008) 5473–5480.

[23] C. Guo, M. Masin, O.S. Qureshi, R.D. Murrell-Lagnado, Evidence for functional P2X4/P2X7 heteromeric receptors, Mol. Pharmacol. 72 (2007) 1447–1456.

[24] O.R. Baricordi, L. Melchiorri, E. Adinolfi, S. Falzoni, P. Chiozzi, G. Buell, F. Di Virgilio, Increased proliferation rate of lymphoid cells transfected with the P2X(7) ATP receptor, J. Biol. Chem. 274 (1999) 33206–33208.

[25] F. Di Virgilio, P. Chiozzi, S. Falzoni, D. Ferrari, J.M. Sanz, V. Venketaraman, O.R. Baricordi, Cytolytic P2X purinoceptors, Cell Death Differ. 5 (1998) 191–199.

[26] F. Di Virgilio, D. Ferrari, E. Adinolfi, P2X(7): a growth-promoting receptor-implications for cancer, Purinergic Signal. 5 (2009) 251–256.

[27] L. Raffaghello, P. Chiozzi, S. Falzoni, F. Di Virgilio, V. Pistoia, The P2X7 receptor sustains the growth of human neuroblastoma cells through a substance P-dependent mechanism, Cancer Res. 66 (2006) 907–914.

[28] E. Adinolfi, M.G. Callegari, D. Ferrari, C. Bolognesi, M. Minelli, M.R. Wieckowski, P. Pinton, R. Rizzuto, F. Di Virgilio, Basal activation of the P2X7 ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth, Mol. Biol. Cell 16 (2005) 3260–3272.

[29] B.D. Humphreys, J. Rice, S.B. Kertesy, G.R. Dubyak, Stress-activated protein kinase/JNK activation and apoptotic induction by the macrophage P2X7 nucleotide receptor, J. Biol. Chem. 275 (2000) 26792–26798.

[30] F.P. Gendron, J.T. Neary, P.M. Theiss, G.Y. Sun, F.A. Gonzalez, G.A. Weisman, Mechanisms of P2X7 receptor-mediated ERK1/2 phosphorylation in human astrocytoma cells, Am. J. Physiol. Cell Physiol. 284 (2003) C571–C581.

[31] Z.A. Pfeiffer, M. Aga, U. Prabhu, J.J. Watters, D.J. Hall, P.J. Bertics, The nucleotide receptor P2X7 mediates actin reorganization and membrane blebbing in RAW 264.7 macrophages via p38 MAP kinase and Rho, J. Leukoc. Biol. 75 (2004) 1173–1182.

[32] S. Pochet, A. Gomez-Munoz, A. Marino, J.P. Dehaye, Regulation of phospholipase D by P2X7 receptors in submandibular ductal cells, Cell Signal. 15 (2003) 927–935.