International Journal of New Technology and Research (IJNTR)
ISSN: 2454-4116, Volume-1, Issue-3, July 2015 Pages 12-16

Novel Peptide Suppresses Gene Expressions of C-MYC and CCND1 in Kasumi-1 Cell Lines

ChiyoMizuochi-Yanagi, Akane Yonehara, Yuka Tanaka, AnthonySwain, Toshiyuki Tsunoda, Senji Shirasawaand, Daisuke Sugiyama

***Abstract*— Leukemias are disorders that cause the abnormal growth and differentiation of hematopoietic cells. Therapies for leukemia are often invasive and there is no guarantee of a positive outcome. Biologically active peptides are being used for a number of therapies for different diseases. We derived several biologically active peptides from fetal liver derived Delta like-1+ hepatoblasts with the intention of finding novel biologically active peptides for the treatment of leukemia. After screening 9 peptides we discovered that two of the peptides caused changes of cell number in the human leukemic cell line Kasumi-1. Further investigation showed that a peptide with the sequence RRRRRRRR(PEG3)CQKKDGPCVINGS also caused suppression of the C-MYC and CCND1 genes at 1 day. The cell number and viability at 1 day along with the gene expression data suggests the peptide is a novel biologically active peptide that will be useful for the investigation of novel therapies for leukemia.**

***Index Terms*— Biologically Active Peptides, Fetal liver, Leukemic cells..**

I. INTRODUCTION

Abnormal deficiencies in the growth and differentiation of hematopoietic cells lead to disorders known as leukemias. In Japan 33.7% of childhood cancer deaths were caused by leukemias in 2013[1]. Therapies such as chemotherapy and radiotherapy are available to treat the different forms of leukemia however relapses are known to occur with 60% of patients succumbing to the disease [2]. The invasive nature of chemotherapy and radiotherapy in addition to the likelihood of relapse indicate that novel therapies that target leukemic cells are of great need.

Biologically active peptides have been recognized as molecules that are useful tools for therapy and the elucidation of drug targets. Biologically active peptides such as TRH, angiotensin, vasopressin, oxytocin, bradykinin were described over years ago and since then, many other such peptides have isolated from mammalian tissues and organs [3]. More recently, other biologically active peptides derived from both animal and plant structures have been identified that play a direct role in hematopoiesis [4],[5],[6].By examining tissues that are directly involved in hematopoiesis we hope to be able to identify novel biologically active peptides that can be used as tools for leukemia therapy.

The Fetal liver (FL) serves as the primary organ for erythroid cell expansion and maturation at mid-gestation. Better understanding of the structures involved in FL hematopoiesis such as the functions of hepatoblasts, endothelial cells and stromal cells are likely to be key in the development of novel therapies for hematopoietic disorders [7]. Previously, our group reported that Delta like-1 (DLK-1)+ hepatoblasts support fetal liver hematopoiesis, particularly erythropoiesis, through EPO, SCF and matrix secretion[8]. Loss of DLK-1+ hepatoblasts in Map2k4-/- mouse embryos resulted in decreased numbers of hematopoietic cells in fetal liver, suggesting a key role of DLK-1+ hepatoblasts in hematopoiesis. When sorted DLK-1+ hepatoblasts were further analyzed by DNA microarray, several genes encoding proteinases and peptidases were highly expressed in DLK-1+ hepatoblasts. Based on the hypothesis that high weight proteins are digested into small we used the DLK-1+ cells to isolate several peptides that could potentially play a role in hematopoietic function.

In this study we have focused on the use of two cell lines; the erythroleukemic cell like K562[9] and the meyleoblastic/monoblastic leukemia cell like Kasumi-1[10]. Both of these cell lines have been identified as useful tools for the in vitro analysis of leukemic cells[11]. Using the biologically active peptides previously described we aimed to screen out novel biologically active peptides that play a role in hematopoiesis and elucidate their effect on disorders of the hematopoietic system.

II. METHODOLOGY

A. Cell culture

The human leukemic cell lines K562 and Kasumi-1 were cultured in RPMI 1640 medium (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS)with10 U/mL penicillin and 10 mg/mL streptomycin (SIGMA-ALDRICH, Saint Louis, MO)at 37°C in 5% CO_2. Cells were cultured in 48-well plates with well volumes of 250µl with $2.5x10^5$ cells/well.

B. Peptide screening and cell counting (add about solubility)

The peptides were provided by the Science Lustre Co., Ltd. (Fukuoka, Japan). The sequences of the screened peptides can

ChiyoMizuicgi-Yanagi, Department of Cell Biology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan,
AkaneYonehara, Center for Clinical and Translational Research, Kyushu University Hospital, Fukuoka, Japan,
Yuka Tanaka, Department of Cell Biology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan,
Anthony Swain, Department of Research and Development of Next Generation Medicine, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan,
Toshiyuki Tsunoda, Department of Cell Biology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan,
SenjiShirasawa, Department of Cell Biology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan,
Daisuke Sugiyama, Center for Advanced Medical Innovation, Kyushu University, Fukuoka, Japan

be found in Table1. The peptides provided were dissolved in H_2O or DMSO based on their solubility and added into culture at a concentration of 10µg/ml. Cell numbers and viabilities were determined by trypan blue staining observation under microscopy. Cells were cultured in single wells during the initial screening and then in triplicate wells for more detailed screening where the mean and standard deviations (SD) were calculated.

Table 1. *Peptides and their sequences provided by Science Lustre Co., Ltd*

Peptide	Sequence
A	RRRRRRRR(PEG3)CQKKDGPCVINGS
B	Ac-RRRRRRRR(PEG3)CQKKDGPCVINGS-NH 2
C	sGnlvcGGdkkqc(PEG3)rrrrrrrr
D	RRRRRRRR-[CQKKDGPCVINGS]-PEG3-NH_2
E	PEG3-[CQKKDGPCVINGS]-NH_2
F	[myristic acid]-[CQKKDGPCVINGS]-NH_2
G	[myristic acid]-[CQKKDGPCVINGS]-PEG3-NH_2
H	PEG3-[CQKKDGPCVINGS]-[lauric acid]-NH_2
I	CQKKDGPCVINGS

C. Quantitative real-time PCR

Total RNA was extracted from each cell lines usingRibopure™ Kit (Life Technologies, Carlsbad, CA). Total RNA was subjected to reverse transcription using a High-Capacity RNA-to-cDNA Kit (Life Technologies) according to established protocols. The mRNA levels of various genes were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Green and gene-specific primers with the StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA). The mRNA level of each target gene was normalized to *β-ACTIN* as an internal control.

D. Immunocytochemistry

The sorted cells were attached onto glass slides (Matsunami glass, Osaka, Japan) by CytoSpin4 (Thermo Fisher scientific, Waltham, MA) at 450 rpm for 7 minutes and air dried. Cells were fixed in 1% paraformaldehyde at 4°C for 30 minutes. After washing with PBS, cells were incubated with PBS containing 0.05% Triton-X 100 at room temperature for 15 minutes. After 3 washes with PBS, the cells were blocked with PBS containing 1% BSA at room temperature for 30 minutes and incubated overnight at 4°C with monoclonal rat anti-mouse Ki-67 primary antibody (DakoCorporation, Glostrup, Denmark). After 3 washes with PBS, cells were incubated with AlexaFluor488 goat anti-rat (1:400, Invitrogen, Carlsbad, CA) and TOTO-3 iodide (1:1500, Invitrogen) at room temperature for 30

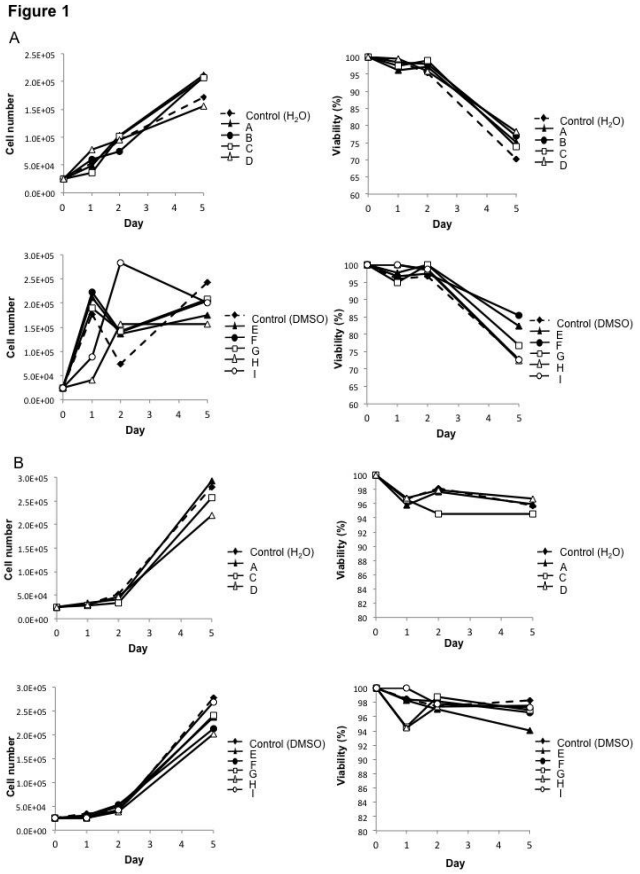

Figure 1. *Proliferation ofhuman cancer cell lines in the presence ofnovel biologically active peptides. K562 cells (A) and Kasumi-1 cells (B) were cultured over a 5-day period with the biologically active peptides in correspondence with Table 1. Cell number and viability was determined by trypan blue staining (n=1).*

minutes. After 3 washes with PBS, cells were mounted on coverslips with fluorescent mounting medium (Dako Corporation) and assessed using a Fluo View 1000 confocal microscope (Olympus, Tokyo, Japan).

III. FINDINGS

A. Effect of co-culturing biologically active peptides with K562 and Kasumi-1 cells.

In order to screen out potential targets for use in the treatment of leukemia we screened 9 peptides (Table 1.) by co-culturing them with the immortalized leukemic cell likes K562 and Kasumi-1. Single culture wells were set and observed over a 5-day period.In K562 cells (Figure 1A.),peptides A, B and C showed an increase in cell number whereas peptides D through to I showed decreased in the number of cells over the culture period. The viability of the cells was increased in peptides A through G but there were no observable differences in peptides H and I. In Kasumi-1 cells (Figure 1B.) only peptide A showed the ability to increase the number of cells with all of the other conditions resulting in lower cell numbers than the control. Peptide D showed an increase in viability over the 5-day period but all of the other peptides showed a decrease in viability with the exception of peptide A that showed now observable differences to the control. Peptides A and D showed the same tendency of increasing

cell number and cell viability over the 5-day period which made them promising candidates for further investigation.

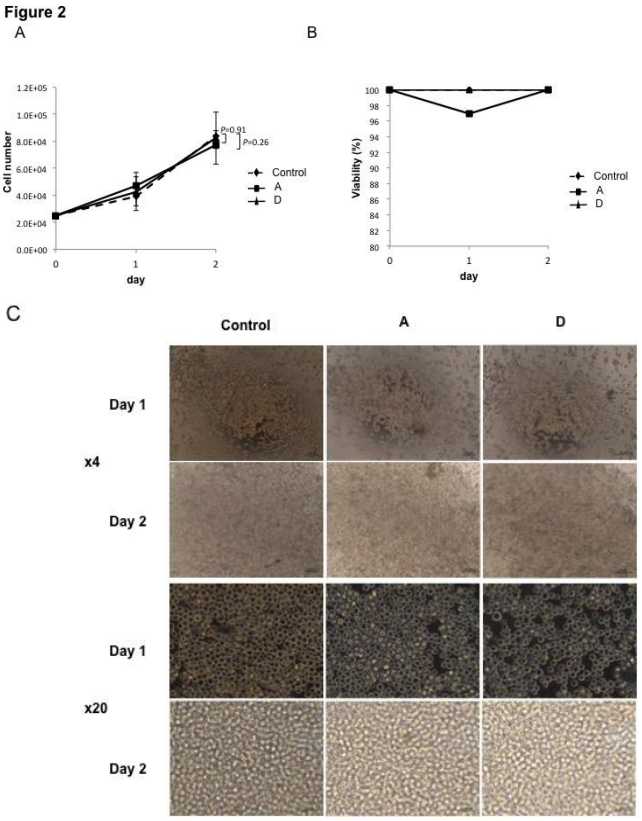

Figure 2. *Proliferation of Kasumi-1 cells in the presence of peptide A and D. Cells were cultured over a 2-day period and the number and viability was determined by trypan blue staining. (A) The number of Kasumi-1 cells at day 1 and day 2 after culture with peptides A and D. (B) The viability of cells at day 1 and day 2 after culture with peptide A and D. (C) Representative images of the culture conditions were taken at day 1 and day 2 at x4 and x20 magnification (n=3).*

B. Peptide A and peptide D increase the number of Kasumi-1 cells at day 1 but not day 2 of culture.

After the initial screening Kasumi-1 cells were cultured in triplicate with peptides A and D (Figure 2A.). At day 1 cells cultured with both peptides showed an increase in cell number with peptide A at 0.21±0.17% ($p=0.28$) and peptide D at 0.10±0.36% ($p=0.72$) increases respectively compared to the control. At day 2 both peptides showed a decrease in cell number compared to the control with peptide A at 0.08±0.03% ($p=0.26$) and peptide D at 0.02±0.23% ($p=0.91$) decreases compared to the control(Figure 2B.). The viability of cells cultured with decreased by 0.08±0.03% ($p=0.26$) with peptide A and 0.02±0.23% ($p=0.91$) with peptide D when compared to the control at day 1 but returned to 100% viability at day 2. Observations of the cell morphology by light microscopy at day 1 and day 2 showed no significant difference in morphology in the presence of peptide A and D compared to the control (Figure 2C.).

C. Peptide A reduces the expression of C-MYC and CCND1 in Kasumi-1 cells at day 1 of culture.

Kasumi-1 cells were cultured with peptide A and D and then analyzed by real time PCR at day 1 and day 2 (Figure 3A.). Culture with peptide A statistically decreased the expression of *C-MYC* by 52.13±1.33% ($p<0.001$) and *CCND1* by 57.80±15.58% ($p<0.001$). At day 2 there was no significant decrease in *C-MYC* expression in either condition but *CCND1* expression was reduced by 26.55±6.36% ($p<0.001$) with peptide A and 12.63%±3.69% ($p<0.005$) with peptide D.

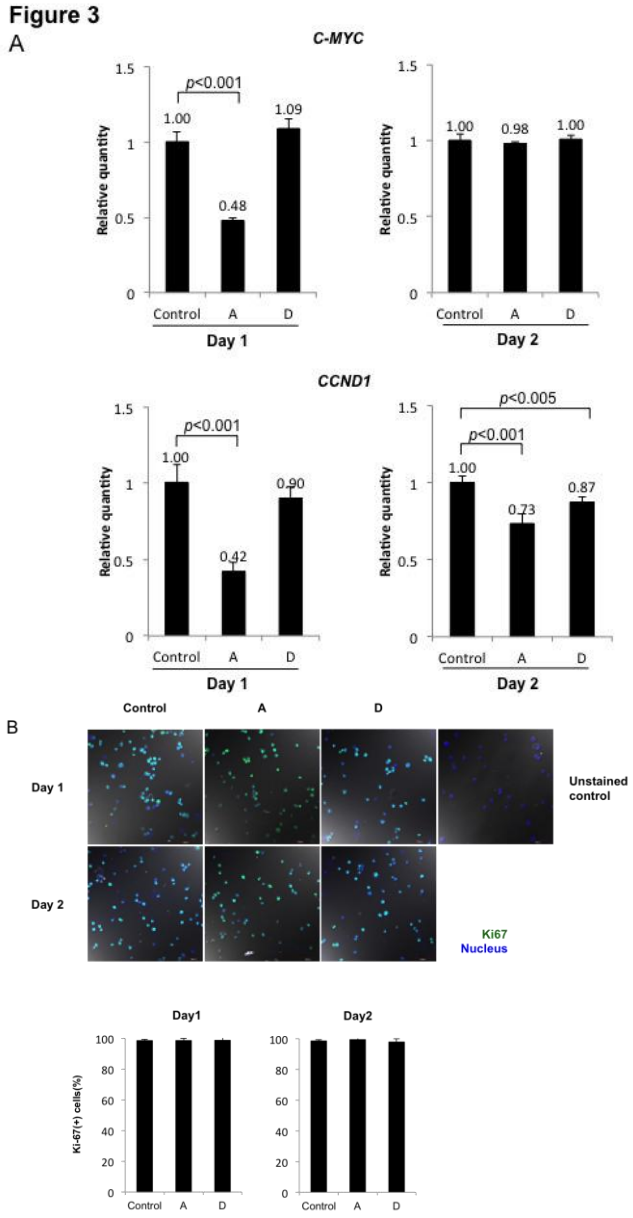

Figure 3. *Gene and protein analysis of Kasumi-1 cells cultured with novel biologically active peptides. (A) Kasumi-1 cells were cultured in the presence of peptide A and peptide D over a 2 day period and the relative expression of C-MYC (upper) and CCND1 (lower) was assessed by real time polymerase chain reaction (PCR) analysis. (B) Representative confocal images of Kasumi-1 cells cultured in the presence of peptide A and peptide D. Ki-67 (green) is localized in the nucleus, which is stained with TOTO-3. Scale bars: 30μM for all panels. The graphs (lower) are numerical representations of the Ki-67 staining (n=3).*

C. Peptide A reduces the expression of C-MYC and CCND1 in Kasumi-1 cells at day 1 of culture.

Kasumi-1 cells were cultured with peptide A and D and then analyzed by real time PCR at day 1 and day 2 (Figure 3A.). Culture with peptide A statistically decreased the expression

of *C-MYC* by 52.13±1.33% ($p<0.001$) and *CCND1* by 57.80±15.58% ($p<0.001$). At day 2 there was no significant decrease in *C-MYC* expression in either condition but *CCND1* expression was reduced by 26.55±6.36% ($p<0.001$) with peptide A and 12.63%±3.69% ($p<0.005$) with peptide D.

D. Both peptide A and peptide D do not affect the expression of Ki-67 in Kasumi-1 cells.

Kasumi-1 cells were cultured in triplicate wells with either peptide A or peptide D for two days. The cells were collected and stained for Ki-67 expression for immunocytochemical analysis (Figure 3B.). Observation of the cells under confocal microscopy showed no difference in the expression of Ki-67 between the control sample and the samples cultured with peptide A and D.

IV. DISCUSSION

After investigation of the novel DLK-1+ derived peptides we were able to make several observations. After initial culture with K562 and Kasumi-1 cells we observed that both peptide A up regulated and peptide D down regulated caused an up-regulation in the number of cells and an increase in the viability of those cells (Figure 1.). Peptides E, F, G, H and I were dissolved in DMSO due to their poor water solubility. The DMSO has been reported to be cytotoxic to leukemic cells at 48 hours which may have affected the cell viability[12].

Further investigation of culture of peptide A and D in Kasumi-1 cells showed up-regulation of both cell number and cell viability (Figure 2). This may be caused by the peptides actions as cell-penetrating peptides (CPP). It was reported that cationic CPP that containthe basic short strands of arginines and lysinescause highly positive net charges which is important for the internalization of certain therapeutic molecules [13]. Both peptide A and peptide D contain long arginine chains compared to the other peptides (Table 1.) which suggests that they are internalized by the cells as cationic CPPs.

Even though both peptides showed an increase in the cell number and viability in Kasumi-1 cells it was only peptide A that showed compatible down regulation of the *C-MYC* and *CCND1*expressions at day 1 (Figure 3A).Oncogenic over expression of *C-MYC*is implicated in the genesis of MYC induced lymphomas[14].Alterations of expression of *CCND1* are also involved in a number of oncogenic mutations with over 100 proteins that interact with the gene already identified [15][16]. The exact mechanism of the interaction of peptide A with the promoter regions of *C-MYC* and *CCND1* is in need of further elucidation.

Both peptide A and peptide D showed no effect on the level of Ki-67 in Kasumi-1 cells (Figure 3B.). The Ki-67 monoclonal antibody that binds to nuclear antigens that has a long history of being used as a tool for selectively identifying proliferating cells [17].Culture with peptide A and peptide D shows that the peptides have no effect on Ki-67 related cell proliferation and therefore influence proliferation by another mechanism.

The duration of bioactivity of peptide A can be elucidated by the reduction of cell viability and subsequent down regulation of *C-MYC* and *CCND1* at 24 hours. Because biologically active peptides are protein structures it is likely that they will begin to degrade after a short period of time. The bioactivity of peptide A could clearly be seen at 24 hours but not beyond that time suggesting that subsequent application of further peptide A at 24 hours may prolong the gene down regulation and reduction in viability of Kasumi-1 cells unless compensatory mechanisms are response for the restoration of viability.

V. CONCLUSION

In conclusion we have elucidate that the peptide with sequence RRRRRRRR(PEG3)CQKKDGPCVINGS known as peptide A is a novel biologically active peptide that suppresses gene expressions of *C-MYC* and *CCND1* in Kasumi-1 cell lines at 1 day. Even though more investing is required into the exact mechanism of this peptide it can be seen as a novel tool for finding targets for leukemia therapy.

ACKNOWLEDGMENT

We thank Dr. KasemKulkeaw and Dr. Tomoko Inoue for their technical support. We would also like to thank Dr. Yoichi Nakanishi and Dr. Kiichiro Nakajima for their support and advice.

REFERENCES

[1] Yang, L. and J. Fujimoto, Childhood cancer mortality in Japan, 1980-2013. BMC Cancer, 2015. 15: p. 446.
[2] Felipe Rico, J., D.C. Hassane, and M.L. Guzman, Acute myelogenous leukemia stem cells: from Bench to Bedside. Cancer Lett, 2013. 338(1): p. 4-9.
[3] Lintner, K. and O. Peschard, Biologically active peptides: from a laboratory bench curiosity to a functional skin care product. Int J Cosmet Sci, 2000. 22(3): p. 207-18.
[4] Nandi, S., M.P. Akhter, M.F. Seifert, X.M. Dai, and E.R. Stanley, Developmental and functional significance of the CSF-1 proteoglycan chondroitin sulfate chain. Blood, 2006. 107(2): p. 786-95.
[5] Queiroz, M.L., M.C. Valadares, C.O. Torello, A.L. Ramos, A.B. Oliveira, F.D. Rocha, V.A. Arruda, and W.R. Accorci, Comparative studies of the effects of Tabebuia avellanedae bark extract and beta-lapachone on the hematopoietic response of tumour-bearing mice. J Ethnopharmacol, 2008. 117(2): p. 228-35.
[6] Zhu, M.X., W.L. Wan, H.S. Li, J. Wang, G.A. Chen, and X.Y. Ke, Thymopentin enhances the generation of T-cell lineage derived from human embryonic stem cells in vitro. Exp Cell Res, 2015. 331(2): p. 387-98.
[7] Swain, A., T. Inoue, K.S. Tan, Y. Nakanishi, and D. Sugiyama, Intrinsic and extrinsic regulation of mammalian hematopoiesis in the fetal liver. Histol Histopathol, 2014. 29(9): p. 1077-82.
[8] Sugiyama, D., K. Kulkeaw, C. Mizuochi, Y. Horio, and S. Okayama, Hepatoblasts comprise a niche for fetal liver erythropoiesis through cytokine production. Biochem Biophys Res Commun, 2011. 410(2): p. 301-6.
[9] Lozzio, C.B. and B.B. Lozzio, Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood, 1975. 45(3): p. 321-34.
[10] Asou, H., S. Tashiro, K. Hamamoto, A. Otsuji, K. Kita, and N. Kamada, Establishment of a human acute myeloid leukemia cell line (Kasumi-1) with 8;21 chromosome translocation. Blood, 1991. 77(9): p. 2031-6.
[11] Inoue, T., A. Swain, Y. Nakanishi, and D. Sugiyama, Multicolor analysis of cell surface marker of human leukemia cell lines using flow cytometry. Anticancer Res, 2014. 34(8): p. 4539-50.
[12] Dennis, A.J. and H.E. Wilson, Altered mitogenic responsiveness of chronic leukemic lymphocytes and normal human lymphocytes treated with dimethyl sulfoxide. Ann N Y Acad Sci, 1975. 243: p. 73-80.
[13] Raucher, D. and J.S. Ryu, Cell-penetrating peptides: strategies for anticancer treatment. Trends Mol Med, 2015.
[14] Dang, C.V., MYC on the path to cancer. Cell, 2012. 149(1): p. 22-35.

[15] Musgrove, E.A., C.E. Caldon, J. Barraclough, A. Stone, and R.L. Sutherland, Cyclin D as a therapeutic target in cancer. Nat Rev Cancer, 2011. 11(8): p. 558-72.
[16] Jirawatnotai, S., Y. Hu, W. Michowski, J.E. Elias, L. Becks, F. Bienvenu, A. Zagozdzon, T. Goswami, Y.E. Wang, A.B. Clark, T.A. Kunkel, T. van Harn, B. Xia, M. Correll, J. Quackenbush, D.M. Livingston, S.P. Gygi, and P. Sicinski, A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. Nature, 2011. 474(7350): p. 230-4.
[17] Gerdes, J., H. Lemke, H. Baisch, H.H. Wacker, U. Schwab, and H. Stein, Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol, 1984. 133(4): p. 1710-5.

ChiyoMizuochi-Yanagi,Education:1996-2000;Kyushu Sangyo University, 2012- present; Fukuoka University Faculty of Medical Sciences (PhD). Work Experience: 2006-2014 SSP Department of Hematopoietic Stem Cells in Kyushu University Faculty of Medical Sciences; Technician.
Current Publications; 1.Sugiyama D, Kulkeaw K, Mizuochi C. TGF-beta-1 up-regulates extra-cellular matrix production in mouse hepatoblasts.Mech Dev. 2013 Feb;130(2-3):195-206. 2. Mizuochi C, Fraser ST, Biasch K, Horio Y, Kikushige Y, Tani K, Akashi K, Tavian M, Sugiyama D. Intra-aortic clusters undergo endothelial to hematopoietic phenotypic transition during early embryogenesis.PLoS One. 2012;7(4):e35763. 3. Sugiyama D, Inoue-Yokoo T, Fraser ST, Kulkeaw K, Mizuochi C, Horio Y.Embryonic regulation of the mouse hematopoietic niche.ScientificWorldJournal. 2011;11:1770-80. 4.Kulkeaw K, Inoue T, Mizuochi C, Horio Y, Ishihama Y, Sugiyama D.Ectopic expression of Hmgn2 antagonizes mouse erythroid differentiation in vitro.Cell Biol Int. 2012 Feb;36(2):195-202.5. Kulkeaw K, Ishitani T, Kanemaru T, Ivanovski O, Nakagawa M, Mizuochi C, Horio Y, Sugiyama D.Cold exposure down-regulates zebrafish pigmentation.Genes Cells. 2011 Apr;16(4):358-67. 6.Sasaki T, Mizuochi C, Horio Y, Nakao K, Akashi K, Sugiyama D.Regulation of hematopoietic cell clusters in the placental niche through SCF/Kit signaling in embryonic mouse.Development. 2010 Dec;137(23):3941-52. 7. Kulkeaw K, Horio Y, Mizuochi C, Ogawa M, Sugiyama D.Variation in hematopoietic potential of induced pluripotent stem cell lines.Stem Cell Rev. 2010 Sep;6(3):381-9. 8.Kulkeaw K, Mizuochi C, Horio Y, Osumi N, Tsuji K, Sugiyama D.Application of whole mouse embryo culture system on stem cell research.Stem Cell Rev. 2009 Jun;5(2):175-80

AkaneYonehara,Education; Faculty of environmental studies, Nagasaki University (BSc). Professional Experience;
2004-2007 Department of Pharmacology and Molecular Therapeutics, Kumamoto University, Technical assistant
2007-2011 LSI Medience Corporation, Technician
2011-2012 Chemicals Evaluation and Research Institute, Japan, Technician
2012-2014 College of Pharmaceutical Sciences, Matsuyama University, Technical assistant
2014- Center for Clinical and Translational Research, Kyushu University Hospital, Technical Staff.
Current publications: 1. Yonehara A, Tanaka Y, Kulkeaw K, Era T, Nakanishi Y, Sugiyama D. Aloe vera extract suppresses proliferation of neuroblastoma cells in vitro.Anticancer research, 2015.

Yuka Tanaka,Education: 2008-2010; Department of Health Sciences, School of Medicine, Kyushu University, 2010-2012;Department of Health sciences, Graduate School of Medical Science, Kyushu University. 2013-present; Department of Cell Biology, Faculty of Medicine, Fukuoka University (PhD).Professional Experience and Research work:
2012-2013 Kyoto prefectural University of Medicine, Technical assistant
2013-2014 Kyushu University Hospital Center for Clinical and Translational Research, Technical Stuff
2014-2015 Center for Advanced Medical Innovation, Kyushu University, Assistant professor
2015-present Japan Society for the Promotion of Science, Research fellow
2015-present JunshinGakuen University, Part-time Lecturer.
Memberships: Japanese Society of Laboratory Medicine
The Japanese Society for Laboratory Hematology
Japanese Society of Legal Medicine
Japanese Society of Hematology
The Society of Biotherapeutic Approaches
Japan Society of Clinical Trials and Research
Licenses: 2010.4 Clinical laboratory technologist
2008.10 Second class Medical Engineering technician
Current Publications:
1. Yonehara A, Tanaka Y, Kulkeaw K, Era T, Nakanishi Y, Sugiyama D. Aloe vera extract suppresses proliferation of neuroblastoma cells in vitro.Anticancer research, 2015.
2. Kojima N, Tanaka Y, Kulkeaw K, Nakanishi Y, Shirasawa S, Sugiyama D. Apoptosis-inducing Factor Mitochondrion-Associated 2, Regulates Kld1 and a Mouse Erythroleukemia Cell Line. Anticancer Research, 2015. (in press)
3 · Keai Sinn Tan, Tomoko Inoue, KulkeawKasem, Yuka Tanaka, Mei I Lai and Daisuke Sugiyama. Localized SCF and IGF-1 secretion enhances erythropoiesis in the spleen of murine embryos. Biology Open, 2015 (in press) 000, 1-12 doi:10.1242/bio.201410686
4 · Tanaka Y, Kulkeaw K, Inoue T, Tan KS, Nakanishi Y, Shirasawa S, Sugiyama D. Dok2 likely down-regulates Klf1 in mouse erythroleukemia cells. Anticancer Research, 34(8): 4561-4568, 2014.
5 · Inoue T, Kulkeaw K, Muennu K, Tanaka Y, Nakanishi Y, Sugiyama D. Herbal drug ninjin'yoeito accelerates myelopoiesis but not erythropoiesis in vitro.Genes to cells, 19(5): 432-440, 2014.