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GAP JUNCTION FUNCTION IS ESSENTIAL FOR SURVIVAL OF ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

Background. Acute lymphoblastic leukemia has an intimate physical relationship with nonmalignant bone marrow stromal cells. We have recently demonstrated that stromal cells contribute to the survival of leukemia cells and that there is a bidirectional transfer of intracellular material between them. Understanding the mechanisms of stromal support of leukemia may provide insights into new therapies. **Aim**. To test the hypothesis that gap junctions are formed between acute lymphoblastic leukemia cells and nonmalignant stromal cells, and that gap junction function is essential for the survival of leukemia cells. **Materials and Methods**. We employed a well-characterized *in vitro* model of human bone marrow stromal cells and primary human B lymphoblastic leukemia cells and measured leukemia cell survival in coculture using flow cytometry. We measured the effects of gap junction antagonist peptides, carbenoxolone (a drug known to interfere with the gap junction function), and several leukemia chemotherapy drugs including methotrexate upon leukemia cell survival. **Results**. We demonstrated that stromal cells need to be alive and metabolically active to keep leukemia cells alive. Physical contact between stromal and leukemia cells leads to an increase in gap junction proteins in leukemia cells. Gap junction inhibitory peptides impaired leukemia cell survival as did carbenoxolone, a nonpeptide inhibitor of the gap junction function. Stromal cell survival was not affected. We observed a very modest enhancement of methotrexate antileukemia activity by low-dose carbenoxolone but no significant interactions with dexamethasone, vincristine, mercaptopurine, or doxorubicin. **Conclusion**. These studies demonstrate that acute lymphoblastic cell survival is impaired by interference with the gap junction function. The development of drugs targeting gap junctions may provide a novel approach to the therapy of acute lymphoblastic leukemia.

Keywords: gap junction, stromal cells, bone marrow, acute lymphoblastic leukemia, carbenoxolone, methotrexate.

Acute lymphoblastic leukemia (ALL) is the most common cancer of childhood. It arises in the bone marrow. While studying malignant cells gives insight into cancer, there is a growing awareness that the microenvironment of nonmalignant cells is also important for cancer. We have recently demonstrated that stroma keeps ALL cells alive and that there is a bidirectional transfer of intracellular material between ALL and stroma [1]. There are several processes by which adjacent cells can exchange

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materials. They include microvesicles, tunneling nanotubules, and gap junctions. As part of our earlier studies, we observed in RNASeq studies that *GJA1*, the gene encoding the gap junction protein connexin 43, was expressed in stromal cells.

Gap junctions are intercellular structures composed of connexins at the cell surface [2]. They allow passage of ions and small molecules of less than 1000 Daltons. They facilitate intercellular signaling induced by cytokines and growth factors. The predominant gap junction in human cells is connexin 43 [3].

It is known that in normal bone marrow, gap junctions are formed between stromal cells and hematopoietic cells and that these may play a role in stromal support of hematopoiesis. However, potential roles are complex with both positive and negative effects on hematopoiesis reported [4—8]. The role of stromal cells in the maintenance of malignant cells is also complex with some observing that gap junctions between AML cells and stromal cells enhance AML survival while others observe that the junctions induce leukemia cell apoptosis [9—11].

In this work, we tested the hypothesis that gap junctions contribute to this stromal support of leukemia cells. We also tested the hypothesis that interference with gap junction function could increase the antileukemia effect of drugs commonly used to treat ALL.

Materials and Methods

Stromal cells. In most experiments, we used a stromal cell line derived from normal bone marrow immortalized with the human telomerase reverse transcriptase (TERT) gene; this cell line was obtained from the lab of Dr. D. Campana [12]. Short-term, primary marrow stromal cultures were frequently used to confirm observations made with the immortalized stromal line. Primary stromal cell cultures were established by placing 1—3 ml of marrow aspirate in 10 mL RPMI supplemented with 20% FCS, MEM non-essential amino acids 1X, sodium pyruvate 1 mM, 2-mercaptopurine 5.5 μM, penicillin/streptomycin 1X, and 1 μM hydrocortisone (R10C+H) in 25 cm^2 conventional tissue culture flasks. (Tissue culture components were purchased from ThermoFisher, USA.) After 24—48 h, nonadherent cells were removed. During the first passage (14—21 days), FCS was reduced to 10% [1]. The term “R10C+H” refers to this complete RPMI tissue culture medium which includes 10% fetal calf serum with supplements and hydrocortisone as described above.

Leukemia cells. Deidentified primary B lineage ALL cells from adult and pediatric patients were obtained from bone marrow or peripheral blood leukapheresis samples at the time of initial diagnosis or relapse. The samples were used under the auspices of an IRB-approved protocol RSRB00046358 Biological Studies of Acute Leukemia, from 2/28/2014 through 9/29/2021. The IRB deemed that individual patient consent was not needed since no personal identifying information was involved and the materials were from residual lab samples that would otherwise be discarded. The researchers did not have access to information that could identify participants during or after data collection. Primary ALL cells were not continuously grown in tissue culture. Rather, ALL patient sample specimens were expanded a single time as patient-derived xenografts in NOD-SCID mice as previously described [1]. Upon growing in the mice, bone marrow and/or spleen cells were harvested and cryopreserved. Aliquots of cryopreserved ALL cells were thawed as needed for use in assays, and over 5 days, the assays were maintained in an AIM V tissue culture medium (ThermoFisher, USA). The work with mice was approved by the University of Rochester Committee on Animal Resources, protocol UCAR-2003-237E/102081 “Targeting microenvironmental support of acute lymphoblastic leukemia”.

Measurement of leukemia cell survival. 5 x 10^3 stromal cells were plated in flat-bottom 96 well plates in R10C+H. Two days later, the medium was removed, and 3 x 10^4 freshly thawed viable ALL cells were added in the AIM V medium (ThermoFisher, USA). Five days later, the wells were harvested, and flow cytometry was performed by a 3-color flow cytometry (huCD19-FITC+ (BD Pharmingen, USA, clone AD2), huCD45-PE dim+ (BD Pharmingen, USA, clone HI30), AAD-, plus 2.5 x 10^5 counting beads). 25,000 bead events were acquired, and the total number of live ALL cells/well was calculated as (number $CD19^+/CD45^{dim}/AAD^-$) x 10. Transwell culture experiments were performed in 12-well plates. Stromal cells (50,000) were plated in tissue culture plates and allowed to adhere overnight. The following day, transwell dish inserts with pore size 0.4 microns (Corning, USA) were placed in some wells. Freshly thawed leukemia cells (100,000) were then added to the wells. Live leukemia cells were counted by flow cytometry 5 days later.

Measurement of stromal cell survival. 5 x 10^3 stromal cells were plated in flat-bottom 96-well plates and allowed to adhere overnight. The next day carbenoxolone was added. Five days later, the number of viable stromal cells was measured using a bioluminescence assay for intracellular ATP (Promega Cell Titer-Glo, USA).

Stromal cell treatment. In some experiments, stromal cells were treated before the addition of leukemia cells. Stromal cells were added to wells and allowed to attach overnight. Some wells with stromal cells were treated with formalin 10% or paraformaldehyde 4% for 20 min at room temperature. Fixative was then removed, and the wells were extensively washed before the addition of leukemia cells. In other experiments, stromal cells were pretreated with a 6-h exposure to 25 μg/mL G418 (ThermoFisher, USA) to inhibit protein synthesis or a 6-h exposure to 1 μM triptolide (InvivoGen, USA) to impair RNA synthesis. After the 6-h exposures, the wells were washed 3 times with a serum-free medium, and then an AIM V medium was added to each well. Leukemia cells were then added to the wells.

Peptides. The Gap27 peptide sequence was SRPTEKTIFII, and the Gap26 peptide sequence was VCYDKSPFISHVR. The scrambled peptide sequences were IEKFISRTIPT and PEITRIKFSIT. OVA peptide sequence was SIINFEKL. Peptide purities were > 90%. Peptides were purchased from Genscript, USA.

Western blots for connexin 43. Immortalized stromal cells were cultured with leukemia cells for 4 days in an AIM V medium. Viable leukemia cells were purified by flow cytometric sorting on human CD19 and AAD. Flask adherent stromal cells were purified by washing away leukemia cells three times with the medium followed by brief treatment with trypsin-LE (Gibco, USA). A proteinase/phosphatase inhibitor cocktail (Cell Signaling, USA, #5872) was added to cells followed by the addition of RIPA buffer (Cell Signaling, USA, #9806). Protein concentrations of samples were measured. Samples were run on polyacrylamide gels (4.5% stacking gel and 10% resolving gel) and transferred to PVDF membranes. Rabbit anti-connexin 43 antibody (Cell Signaling, USA, #3512) was used for Cx43 detection. Rabbit anti-tubulin antibody (Cell Signaling, USA, #2144) was used in controls. Goat anti-rabbit IgG HRP-linked antibody (Thermo Scientific, USA, #31460) and chemiluminescent substrates (Thermo Scientific, USA, #34580) were used for antibody detection.

Drug treatment in vitro. Carbenoxolone (Thermo Scientific, USA) was diluted in sterile water to make 150 mM stock solutions, which were then diluted in an AIM V medium for addition to cell culture wells. Carbenoxolone was added to leukemia/stromal cell cultures at the time leukemia cells were added to wells. After 5 days of drug exposure, leukemia cell viability was measured by flow cytometry as described above.

Statistics. Statistical analyses and graph production were performed using R statistical language and RStudio. The data from leukemia cell survival assays was found to be normally distributed. Parametric *t*-tests were performed. Graphs are conventional box-and-whiskers plots with the horizontal line representing the median, the box representing the interquartile range, and the whiskers representing the highest and lowest values (outliers excluded).

Results

Physical contact between leukemia cells and stromal cells is necessary for leukemia cell survival. We and others have observed that human ALL cells typically undergo apoptosis *in vitro* but do survive when cocultured with bone marrow stromal cells. The reasons for this are not fully understood. We conducted experiments asking whether physical contact between stromal cells and leukemia cells is necessary for leukemia cell survival. Leukemia cells were placed in three types of wells: wells without stroma, wells with stroma, and transwells with stromal cells on the well bottom above which a porous membrane was placed that allowed diffusion of stromal cell secreted molecules but prevented leukemia cells from directly contacting the stromal cells. Fig. 1, *a* demonstrates that leukemia cells in physical contact with stroma survived (middle column), while poor leukemia cell survival is seen in the transwells (right column).

Stromal cells must be alive and capable of protein synthesis to promote leukemia cell survival. We then

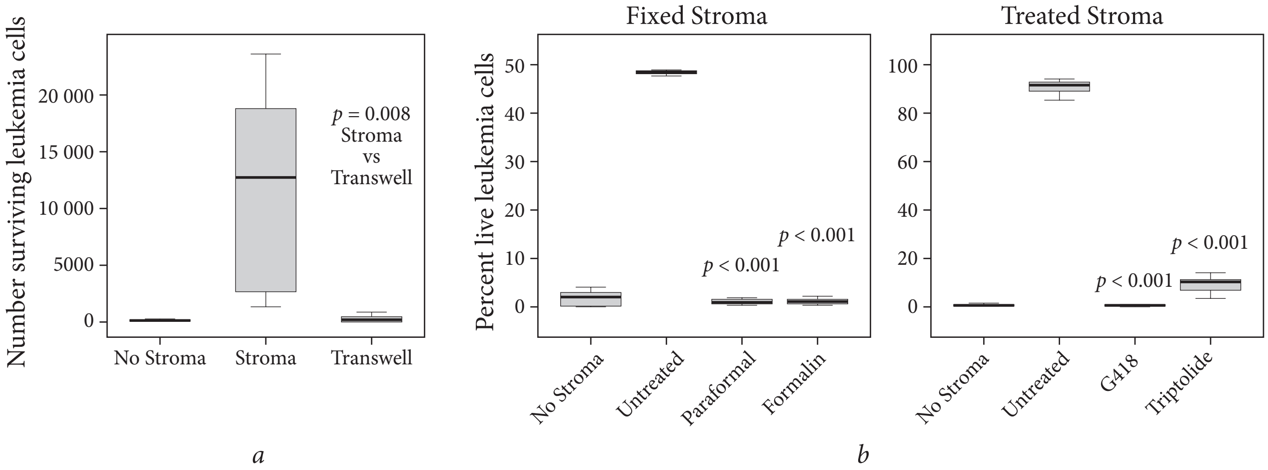
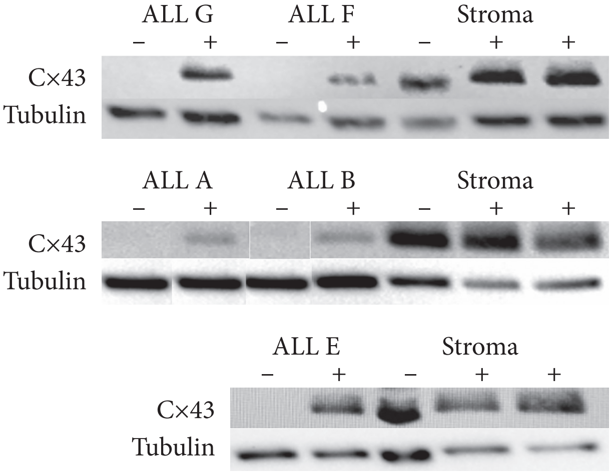


Fig. 1. Leukemia cells survive when in direct contact with live and metabolically active cells. (*a*) Direct stromal cell contact is necessary for leukemia cell survival. Leukemia cells were placed in wells without stroma (“NoStroma”), with stromal cells on the plate (”Stroma”), or on top of porous transwell membranes (”Transwell”) under which were stroma. There were 8 replicates in each condition. (*b*) Leukemia cell survival is impaired when stromal cells are treated with fixatives or inhibitors of protein or RNA synthesis. The left panel shows the percent of live leukemia cells in wells without stroma, untreated stroma, and stroma pretreated with paraformaldehyde or formalin. *p* values reflect comparison with the untreated stroma group (n = 12 in each group). The right panel shows the percent of live leukemia cells in wells without stroma, with untreated stroma, or with stroma pretreated with either G418 or triptolide. Data are presented in box-and-whisker plots. *p* values reflect comparison with the untreated stroma group (n = 16 in each group)



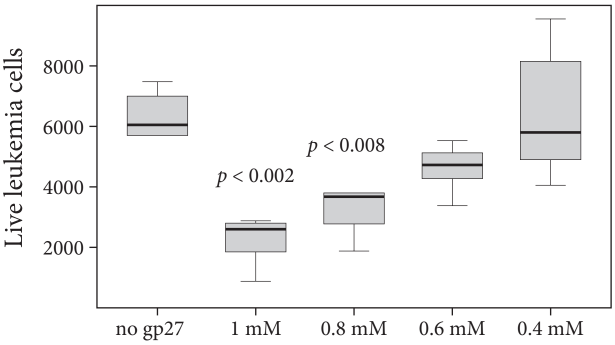


Fig. 2. Western blots demonstrate the presence of connexin 43 in leukemia cells after 4 days of coculture with stromal cells. Cx43 denotes a blot performed with anti-connexin 43 antibody. Tubulin is the control blot using anti-tubulin antibody. “–“ indicates that the sample was from leukemia or stromal cells that had not been cocultured. “+” indicates the sample obtained from flow cytometrically purified leukemia or stromal cells after 4 days of coculture

Fig. 3. Dose-response study of the effect of peptide Gap27 on leukemia cell survival. The number of live leukemia cells after 5 days of coculture with stromal cells. Data are presented in box-and-whisker plots. *p* values reflect the comparison with controls (no Gap27 (“gp27”)). n = 6 for each condition

asked whether the stroma needs to be alive to promote leukemia cell survival. We compared the survival of leukemia cells on untreated stroma and stroma treated in three different ways (Fig. 1, *b*). First, stroma cells were fixed with formalin, essentially leaving intact the cell membrane structures but completely devitalizing the stroma. Leukemia cells did not survive on fixed stroma. Second, stroma cells were pretreated with a sublethal dose of G418, a protein synthesis inhibitor. Stroma

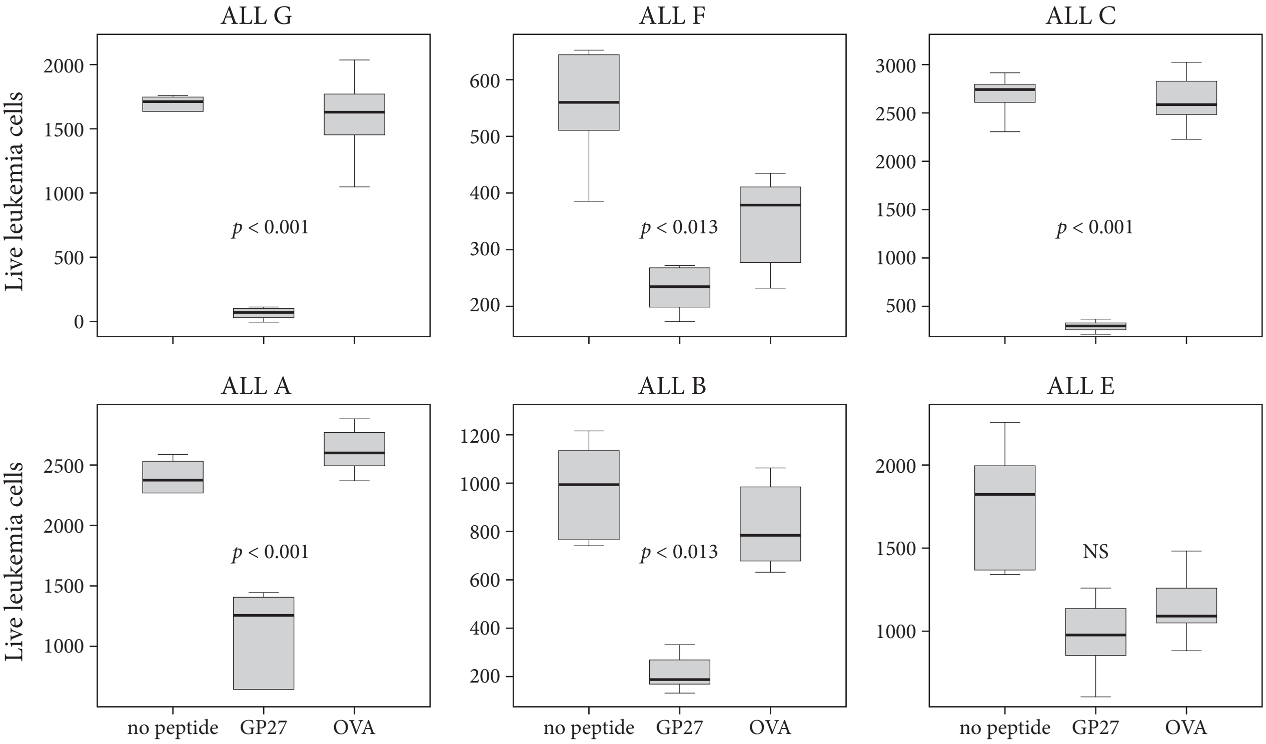


Fig. 4. Gap27 peptide impairs leukemia cell survival. The six figures present the number of live leukemia cells recovered from wells with stroma in the absence or presence of the peptide. Each figure represents leukemia from a different patient. GP27 signifies 1 mM of gap27 peptide while OVA signifies 1 mM of O peptide. Data are presented in box-and-whisker plots. P values reflect the comparison of GP27 with OVA. n = 6 for each condition

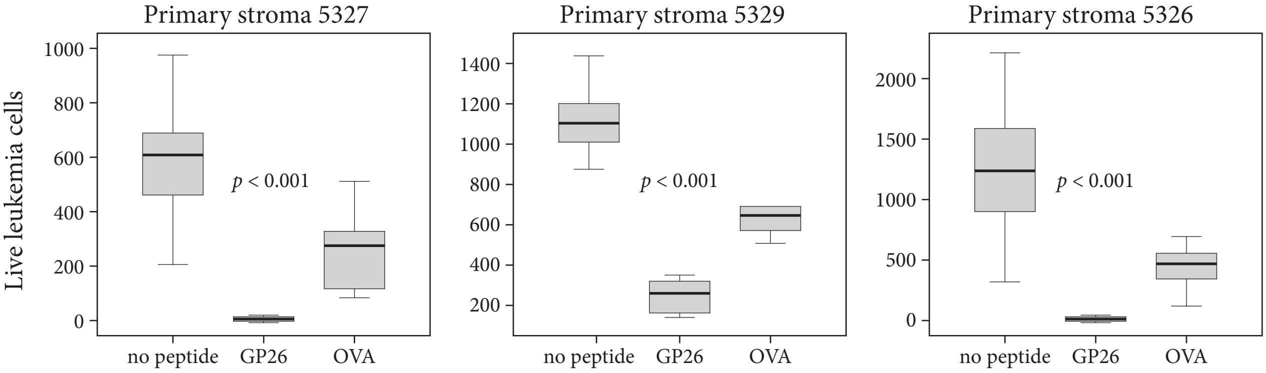


Fig. 5. Gap26 peptide impairs leukemia cell survival. The three figures present the number of live leukemia cells recovered from wells with primary bone marrow stroma in the absence or presence of the peptide. GP26 signifies 1 mM of gap26 peptide, and OVA signifies 1 mM of Ova peptide. Data are presented in box-and-whisker plots. P values reflect the comparison of the GP26 group with the OVA group. n = 6—12 for each condition

cells not synthesizing protein did not promote leukemia cell survival. Third, stroma cells were pretreated with triptolide, an inhibitor of RNA synthesis. Leukemia cell survival was significantly reduced on triptolide-treated stroma.

Gap junctions are formed between leukemia cells and stromal cells. The need for contact and the need for the stromal cells to be alive and transcriptionally active suggested that intercellular transport of materials was important for leukemia cell survival. Gap junctions are used by many cell types for intercellular transport. We performed Western blot experiments to assess the presence of connexin 43, the most common component of human gap junctions, on leukemia and stromal cells (Fig. 2). The experiments were performed on both leukemia and stromal cells before and after coculture of leukemia with stroma. Stromal cells expressed connexin 43 in the absence and presence of leukemia cells. We were unable to detect connexin 43 in leukemia

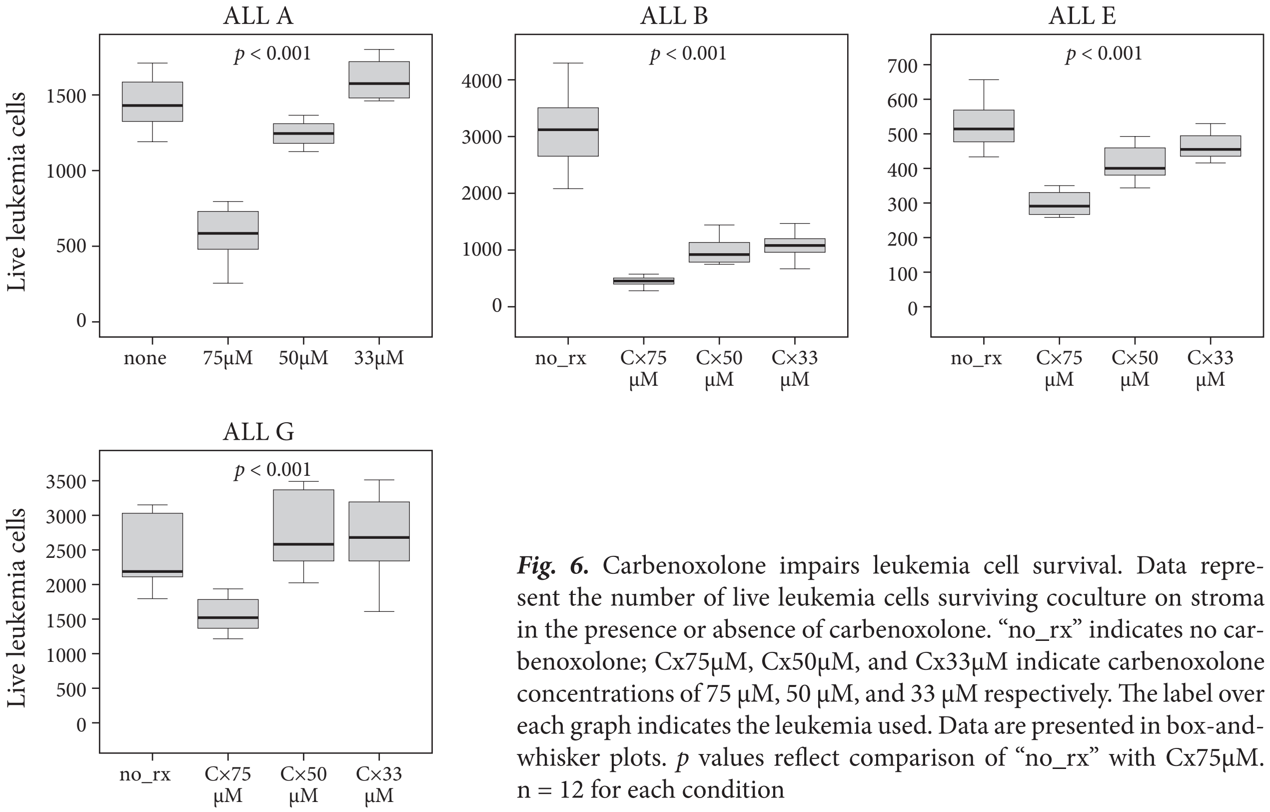


Fig. 6. Carbenoxolone impairs leukemia cell survival. Data represent the number of live leukemia cells surviving coculture on stroma in the presence or absence of carbenoxolone. "no_rx" indicates no carbenoxolone; Cx75µM, Cx50µM, and Cx33µM indicate carbenoxolone concentrations of 75 µM, 50 µM, and 33 µM respectively. The label over each graph indicates the leukemia used. Data are presented in box-and-whisker plots. p values reflect comparison of "no_rx" with Cx75µM. n = 12 for each condition

cells before their incubation with stroma. However, after four days of coculture, all leukemia cells purified by flow sorting expressed connexin 43. We examined the transcription of *GJA1*, the gene encoding connexin 43, in both leukemia cells and stromal cells. *GJA1* was transcribed in both cell types, albeit at about 10-fold greater levels in stromal cells. After contact with stroma, *GJA1* transcription increased in two samples of the leukemia cells but not the others (data not shown).

Peptides that interfere with gap junctions lead to leukemia cell death. We performed experiments determining if peptides that inhibit connexin 43 containing gap junctions would affect leukemia cell survival [13, 14]. Gap27 is a peptide (SRPTEKTIFII) that targets the extracellular domain of connexin 43. It was added to stromal cell/leukemia cultures at a range of concentrations. Fig. 3 shows that Gap27 at 1 mM and 0.8 mM significantly reduced leukemia cell survival.

The inhibitory effect of Gap27 was reproducible in our panel of leukemias. We assessed the specificity of the effect by comparing it to the effect of a peptide derived from ovalbumin (OVA) and demonstrated statistical significance in 5 of the 6 leukemia samples tested (Fig. 4).

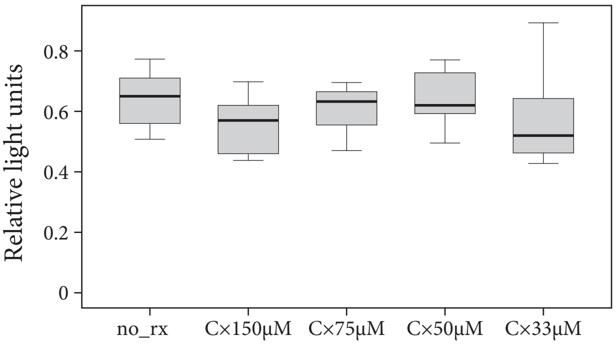


Fig. 7. Carbenoxolone does not impair stromal cell viability. The figure presents stromal viability after 5 days of incubation in carbenoxolone. Viability was assessed in a luminescence assay. "no_rx" indicates no carbenoxolone control. Cx150µM, Cx75µM, Cx50µ, and Cx33µM represent carbenoxolone concentrations of 150 µM, 75 µM, 50 µM, and 33 µM respectively. Data are presented in box-and-whisker plots; t-test comparisons were made between "no_rx" and each group. There were no statistically significant differences. n = 6 for each condition

Gap26 is another peptide that targets another extracellular domain of connexin 43. When added to leukemia/primary bone marrow stromal cell cocultures, it also produced significant and specific reductions in leukemia cell survival (Fig. 5).

Carbenoxolone impairs leukemia cell survival but not stromal cells. Carbenoxolone is a non-peptide inhibitor of gap junction function, which is widely used in neurobiology research [15]. We added it to leukemia/stromal cell cocultures to determine if it would affect leukemia cell survival. We found that carbenoxolone at 75 μM significantly reduced leukemia cell survival (Fig. 6). We also assessed the effect of carbenoxolone on the survival of stromal cells alone and found no impact of carbenoxolone on the viability of immortalized stromal cells (Fig 7).

Carbenoxolone enhances the toxicity of methotrexate for leukemia cells. Our finding that reduction in leukemia cell survival was produced by inhibition of the gap junction function by either peptides or the drug carbenoxolone led us to ask whether inhibition of the gap junction function could enhance the antileukemia effect of chemotherapy drugs commonly used to treat ALL. We used carbenoxolone in these studies because it was developed many years ago as a drug for peptic ulcers and had been used in humans. We examined its influence on the antileukemia effect of the following chemotherapy drugs: dexamethasone, vincristine, doxorubicin, asparaginase, 6-mercaptopurine, and methotrexate. We performed dose-response studies for the drugs to identify concentrations that would induce alone measurable but minimal antileukemia effects. Carbenoxolone was added to leukemia/stromal cell cocultures along with the individual drugs, and leukemia cell survival was measured. Carbenoxolone did not significantly increase the antileukemia effects of any of the drugs other than methotrexate; there was some very modest enhancement of the methotrexate effect by carbenoxolone (data not shown).

Discussion

We observed that living and transcriptionally active stromal cells can keep ALL alive if they are in physical contact with leukemia cells. We observed that connexin 43 gap junction protein was substantially increased in ALL cells following coculture with stromal cells and that stromal cells constitutively expressed connexin 43.

Interference with gap junction function between ALL cells and stromal cells led to ALL cell death. Physical interference with gap junctions using the gap-junction-specific peptides gap27 and gap26 resulted in significant increases in ALL cell death. Pharmacologic interference with gap junction function with carbenoxolone also led to significant increases in leukemia cell death. Of interest, the concentrations at which carbenoxolone was active in our system were similar to the plasma levels of carbenoxolone observed in humans taking carbenoxolone [16, 17]. In contrast, carbenoxolone did not affect stromal cell viability.

We did not observe additive or synergistic effects with carbenoxolone and many commonly used conventional anticancer drugs active in ALL. This finding in ALL is contrary to those recently reported on acute myelogenous leukemia. Kouzi et al. [18] observed that stromal cells protected AML cells from cytarabine and that disruption of gap junctions by carbenoxolone reduced this protection. Sinyuk et al. [19] observed that disruption of the gap junction component connexin 25 (Cx25) but not connexin 43 enhanced AML sensitivity to cytarabine. We did not study cytarabine in our model system.

Gap junctions have also been studied in other cancers, and complex roles have been seen. Inhibition of gap junctions has been reported to enhance melanoma proliferation [20]. In contrast, gap junction function may enhance the growth of brain metastasis from breast and lung cancers [21]. Enhancement of gap junction function in mesothelioma cells has been shown to increase the antitumor effects of sunitinib [22]. Similar observations have been made with proteasome inhibitors [23]. Gap junctions have also been shown to enhance bystander killing of tumor cells by the chemotherapy drug cisplatin [24]. Upregulation of gap junction function has been shown to increase neuroblastoma cell sensitivity to doxorubicin [25].

Our studies have limitations. First, they were conducted *in vitro*, not *in vivo*. Second, we did not test the effect of pharmacologic gap junction disruption in normal hematopoietic progenitor cells. Future studies exploring gap junction antagonists as drugs for ALL would need to address both of these limitations to assess the critical question of whether such agents could produce relatively selective antileukemia effects without unacceptable hematopoietic or other systemic toxicity.

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Availability of data and materials

The data and materials will be made available upon request.

Authors' contributions

Elena Edwards performed experiments and data analysis and edited the manuscript. Dominic Schenone performed experiments and data analysis and edited the manuscript. Urmila Sivagnanalingam performed experiments and data analysis and edited the manuscript. Sheldon Perry performed experiments, data analysis, and edited the manuscript. Craig A. Mullen was responsible for the overall planning and direction of the experiments, performed data analysis, prepared the manuscript, and obtained funding for the project.

Ethics

Tissue samples were used under the auspices of IRB-approved protocol from 2/28/2014 through 9/29/2021.

Patient consent

The IRB deemed that individual patient consent was not needed since no personal identifying information was involved and the materials were from residual lab samples that would otherwise have been discarded.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Usmani S, Sivagnanalingam U, Tkachenko O, et al. Support of acute lymphoblastic leukemia cells by nonmalignant bone marrow stromal cells. *Oncol Lett*. 2019;17(6):5039-5049. https://doi.org/10.3892/ol.2019.10188
2. Grosely R, Kopanic JL, Nabors S, et al. Effects of phosphorylation on the structure and backbone dynamics of the intrinsically disordered connexin43 C-terminal domain. *J Biol Chem*. 2013;288(34):24857-24870. https://doi.org/10.1074/jbc.M113.454389
3. Kim MO, Lee YJ, Han HJ. Involvement of Cx43 phosphorylation in 5'-N-ethylcarboxamide-induced migration and proliferation of mouse embryonic stem cells. *J Cell Physiol*. 2010;224(1):187-194. https://doi.org/10.1002/jcp.22117
4. Gonzalez-Nieto D, Li L, Kohler A, et al. Connexin-43 in the osteogenic BM niche regulates its cellular composition and the bidirectional traffic of hematopoietic stem cells and progenitors. *Blood*. 2012;119(22):5144-5154. https://doi.org/10.1182/blood-2011-07-368506
5. Dürig J, Rosenthal C, Halfmeyer K, et al. Intercellular communication between bone marrow stromal cells and CD34+ haematopoietic progenitor cells is mediated by connexin 43-type gap junctions. *Br J Haematol*. 2000;111(2):416-425. https://doi.org/10.1046/j.1365-2141.2000.02385.x
6. Rosendaal M, Krenács TT. Regulatory pathways in blood-forming tissue with particular reference to gap junctional communication. *Pathol Oncol Res*. 2000;6(4):243-249. https://doi.org10.1007/bf03187326
7. Cancelas JA, Koevoet WL, de Koning AE, et al. Connexin-43 gap junctions are involved in multiconnexin-expressing stromal support of hemopoietic progenitors and stem cells. *Blood*. 2000;96(2):498-505. PMID: 10887111
8. Rosendaal M, Green CR, Rahman A, Morgan D. Up-regulation of the connexin43+ gap junction network in haemopoietic tissue before the growth of stem cells. *J Cell Sci*. 1994;107(Pt 1):29-37. https://doi.org/10.1242/jcs.107.1.29
9. Singh AK, Cancelas JA. Gap junctions in the bone marrow lympho-hematopoietic stem cell niche, leukemia progression, and chemoresistance. *Int J Mol Sci*. 2020;21(3). https://doi.org/10.3390/ijms21030796
10. Yang S, Wen Q, Liu Y, et al. Increased expression of CX43 on stromal cells promotes leukemia apoptosis. *Oncotarget*. 2015;6(42):44323-44331. https://doi.org/10.18632/oncotarget.6249
11. Zhang X, Liu Y, Si YJ, et al. Effect of Cx43 gene-modified leukemic bone marrow stromal cells on the regulation of Jurkat cell line in vitro. *Leuk Res*. 2012;36(2):198-204. https://doi.org/10.1016/j.leukres.2011.10.001
12. Mihara K, Imai C, Coustan-Smith E, et al. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. *Br J Haematol*. 2003;120(5):846-849. https://doi.org/10.1046/j.1365-2141.2003.04217.x
13. Rajnai H, Teleki I, Kiszner G, et al. Connexin 43 communication channels in follicular dendritic cell development and in follicular lymphomas. *J Immunol Res*. 2015;2015:528098. https://doi.org/10.1155/2015/528098
14. Tarzemany R, Jiang G, Larjava H, Hakkinen L. Expression and function of connexin 43 in human gingival wound healing and fibroblasts. *PloS One*. 2015;10(1):e0115524. https://doi.org/10.1371/journal.pone.0115524
15. Moore AR, Zhou WL, Sirois CL, et al. Connexin hemichannels contribute to spontaneous electrical activity in the human fetal cortex. *Proc Natl Acad Sci U S A*. 2014;111(37):E3919-E3928. https://doi.org/10.1073/pnas.1405253111
16. Pinder RM, Brogden RN, Sawyer PR, et al. Carbenoxolone: a review of its pharmacological properties and therapeutic efficacy in peptic ulcer disease. *Drugs*. 1976;11(4):245-307. https://doi.org/10.2165/00003495-197611040-00002

17. Downer HD, Galloway RW, Horwich L, Parke DV. The absorption and excretion of carbenoxolone in man. *J Pharm Pharmacol*. 1970;22(7):479-487. https://doi.org/10.1111/j.2042-7158.1970.tb10550.x
18. Kouzi F, Zibara K, Bourgeais J, et al. Disruption of gap junctions attenuates acute myeloid leukemia chemoresistance induced by bone marrow mesenchymal stromal cells. *Oncogene*. 2020;39(6):1198-1212. https://doi.org/10.1038/s41388-019-1069-y
19. Sinyuk M, Alvarado AG, Nesmiyanov P, et al. Cx25 contributes to leukemia cell communication and chemosensitivity. *Oncotarget*. 2015;6(31):31508-31521. https://doi.org/10.18632/oncotarget.5226
20. Tittarelli A, Guerrero I, Tempio F, et al. Overexpression of connexin 43 reduces melanoma proliferative and metastatic capacity. *Br J Cancer*. 2015;113(2):259-267. https://doi.org/10.1038/bjc.2015.162
21. Chen Q, Boire A, Jin X, et al. Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature*. 2016;533(7604):493-498. https://doi.org/10.1038/nature18268
22. Uzu M, Sato H, Yamada R, Kashiba T, Shibata Y, Yamaura K, Ueno K. Effect of enhanced expression of connexin 43 on sunitinib-induced cytotoxicity in mesothelioma cells. *J Pharmacol Sci*. 2015;128(1):17-26. https://doi.org/10.1016/j.jphs.2015.04.002
23. Huang T, Zhu Y, Fang X, et al. Gap junctions sensitize cancer cells to proteasome inhibitor MG132-induced apoptosis. *Cancer Sci*. 2010;101(3):713-721. https://doi.org/10.1111/j.1349-7006.2009.01421.x
24. Jensen R, Glazer PM. Cell-interdependent cisplatin killing by Ku/DNA-dependent protein kinase signaling transduced through gap junctions. *Proc Natl Acad Sci U S A*. 2004;101(16):6134-6139. https://doi.org/10.1073/pnas.0400051101
25. Carystinos GD, Alaoui-Jamali MA, Phipps J, et al. Upregulation of gap junctional intercellular communication and connexin 43 expression by cyclic-AMP and all-trans-retinoic acid is associated with glutathione depletion and chemosensitivity in neuroblastoma cells. *Cancer Chemother Pharmacol*. 2001;47(2):126-132. https://doi.org/10.1007/s002800000231

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ФУНКЦІОНУВАННЯ ЩІЛИННИХ КОНТАКТІВ Є СУТТЄВИМ ДЛЯ ВИЖИВАННЯ КЛІТИН ГОСТРОЇ ЛІМФОБЛАСТНОЇ ЛЕЙКЕМІЇ

Стан питання. Лейкемічні клітини гострої лімфобластної лейкемії фізично щільно взаємодіють з незлоякісними стромальними клітинами кісткового мозку. Раніше ми показали, що стромальні клітини є важливими для виживання лейкемічних клітин, а також, що між ними існує двоспрямований перенос внутрішньоклітинного матеріалу. Розуміння механізмів стромальної підтримки лейкемічних клітин може дати поштовх до розробки нових методів лікування лейкемії. **Мета**. В роботі перевірено гіпотезу щодо важливості формування щілинних контактів між клітинами гострої лімфобластної лейкемії та незлоякісними стромальними клітинами кісткового мозку для виживання лейкемічних клітин. **Матеріали та методи**. На моделі стромальних клітин кісткового мозку людини та первинної культури В-лімфобластних лейкемічних клітин методом проточної цитометрії досліджували виживаність лейкемічних клітин за їх взаємного культивування. Вивчали вплив карбеноксолону — антагоністу пептидів щілинних контактів та кількох хіміотерапевтичних засобів, що застосовуються для лікування лейкемії, включаючи метотрексат, на виживаність лейкемічних клітин. **Результати**. Для підтримки функціонування лейкемічних клітин потрібні живі і метаболічно активні стромальні клітини. Фізичний контакт між стромальними і лейкемічними клітинами приводить до збільшення вмісту білків щілинних контактів в лейкемічних клітинах. Інгібітор пептидів щілинних контактів карбеноксолон негативно впливає на виживання лейкемічних клітин, не впливаючи при цьому на стромальні клітини. Низькі дози карбеноксолону лише обмежено підсилюють протилейкемічну активність метотрексату. Взаємодії між ефектами карбеноксолону з одного боку та протилейкемічними препаратами вінкристином, меркаптопурином або доксорубіцином не виявлено. **Висновки**. Інгібування щілинних контактів зменшує виживання лейкемічних клітин гострої лімфобластної лейкемії. Розробка засобів, що впливають на щілинні контакти, може стати перспективним підходом для лікування гострої лімфобластної лейкемії.

Ключові слова: щілинні контакти, стромальні клітини, кістковий мозок, гостра лімфобластна лейкемія, карбеноксолон, метотрексат.