Structural Fragility of Blood Vessels and Peritoneum in Calponin h1-deficient Mice, Resulting in an Increase in Hematogenous Metastasis and Peritoneal Dissemination of Malignant Tumor Cells

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ABSTRACT

We have observed weak expression of calponin h1, which stabilizes the actin filament system, in blood vessels within human malignant tumors. This observation suggested that because of a deficiency in stabilization by calponin h1, the structure of blood vessels in malignant tumors is fragile compared with blood vessels in normal tissues. We therefore generated calponin h1-deficient (CN⁻/⁻) mice to examine the effect of calponin h1 on the integrity of the barrier system in blood vessels against cancer metastasis. The CN⁻/⁻ mice exhibited morphological fragility of the tissues, including the uterine and blood vessels. In particular, we frequently observed bleeding into the surrounding tissue from blood vessels of the ocular fundus in CN⁻/⁻ mice. In addition, mesothelial cells, which usually express calponin h1 in normal (CN⁺/+/⁻) mice, were retracted in the CN⁻/⁻ mice. When fluorescein was injected i.v. into mice, the CN⁻/⁻ mice exhibited a greater and more rapid leakage of fluorescein from the blood vessels of the ocular fundus compared with the CN⁺/+/⁻ mice. In the CN⁻/⁻ mice receiving i.v. inoculations of B16 melanoma cells, significantly more metastatic nodules were formed in the lung than in the CN⁺/+/⁻ mice. When B16 melanoma cells were injected i.p., the severity of peritonitis carcinomatosa was greater in CN⁻/⁻ than in CN⁺/+/⁻ mice. These results indicate that calponin h1 plays an important role in the regulation of the integrity of the blood vessels and peritoneum, which in turn is an important factor influencing the frequency of cancer metastasis. The CN⁻/⁻ mice, which exhibit fragile blood vessels and peritoneum, could serve as sensitive and useful host models to investigate cancer metastasis.

INTRODUCTION

Cancer metastasis consists of multiple steps. Recent molecular biological approaches have greatly assisted in the identification and characterization of numerous molecules responsible for the metastatic phenotypes of cancer cells, such as proteases and cell adhesion molecules. In particular, we have concentrated on the cytoskeletal molecules, including actin (1–3), vinculin (4), tropomyosin (5, 6), calponin h1 (7, 8), and keratins (9), which play important roles in the regulation of cellular morphology, adhesiveness, motility, and/or growth. Because cancer metastasis occurs as a result of the interaction between cancer cells and the host, we have also directed our attention to the host factors relating to cancer metastasis in addition to the phenotypes of tumor cells.

Calponin h1 is an actin-binding protein, largely expressed in smooth muscle cells, that positively regulates and stabilizes actin polymerization and inhibits actomyosin ATPase (10). Calponin h1 has also been detected in the endothelial cells of blood vessels by RT-PCR, although the expressed amount is low compared with that in smooth muscle cells (11). Three calponin isoforms, calponin h1 and h2 and acidic calponin, have been identified and characterized (12, 13). Calponin h1 is expressed mainly in the smooth muscle cells, whereas calponin h2 is a nonmuscle type and acidic calponin is present mainly in the brain. The differences in the biological functions among these isoforms remain to be determined. Calponin h1 is suppressed in transformed smooth muscle cells in leiomyosarcoma (7). Introduction of the calponin h1 gene into the transformed cells suppressed proliferation and induced the differentiated phenotype (8).

We previously reported the down-regulation of αSMA in malignant human melanoma tissues compared with benign pigment tissues (14). When tumor tissues were immunologically stained with anti-αSMA, the expression of αSMA was decreased in the blood vessels of malignant tumors (15–17). We also observed that a melanoma cell line, M14, released PDGF-BB, a product of the sis oncogene, which suppressed αSMA. These observations indicated to us that the low expression of αSMA in the vessels of malignant tumors is induced by the paracrine effect of factors excreted from the tumor cells. Additionally, in the blood vessels of several malignant human tumors, we observed that the down-regulation of calponin h1, which is also induced by PDGF-BB (18), as seen with αSMA, was greater than that of αSMA: calponin h1 expression was not detected, even in blood vessels expressing αSMA. We then assumed that a reduction in calponin h1, as well as in αSMA, leads to fragile blood vessels and the subsequent enhancement of cancer metastasis because the suppression of calponin h1 results in destabilization of actin filaments, which probably weakens the cellular adhesion molecular mechanisms, such as the cadherin and integrin systems, that are regulated in connection with actin filaments.

In the present study, we generated calponin h1-deficient (CN⁻/⁻) mice and examined the degree of sensitivity of these mice to cancer metastasis. We also examined the morphology of several tissues usually rich in calponin h1-positive cells and assessed the permeability of blood vessels together with the degree of hematogenous metastasis and peritoneal dissemination of cancer cells in CN⁻/⁻ mice.

MATERIALS AND METHODS

CN⁻/⁻ Mice. CN⁻/⁻ mice were generated as described previously (19). Targeting of the calponin h1 gene was achieved by use of ES cells from 129SV/J mice, and chimeric mice were generated by injecting the ES cells into C57BL6/J blastocysts. Offspring exhibiting germline transmission were interbred to generate homozygous mice. The mice thus generated were mated with C57BL6/J mice to increase the genetic similarity of the offspring to C57BL6/J mice.

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4 The abbreviations used are: RT-PCR, reverse transcription-PCR; αSMA, smooth muscle α-actin; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.
mice, in which B16 melanoma cells are transplantable. The genotypes of the mice used were determined by Southern blot analysis and/or PCR.

For the PCR, exons 4 and 5 of the calponin h1 gene were amplified to examine deletion of the fragment from the EcoRV site in the intronic site of exon 4–5 to the Apal site of exon 7, using the primers 5’-CATACACAGTTCATGGCAC-3’ (forward) and 5’-TCTTGCTTCTTCAACTCTC-3’ (reverse), the combination of which generated 297-bp DNA fragments. To confirm the replacement of the EcoRV-Apal fragment with the neo-containing cassette, the neo-resistant gene was amplified using primers 5’-GGGACACAGCAGAGGCGCT-3’ (forward) and 5’-ACTTCGCAACTAGGCAGCA-3’ (reverse), which generated a 218-bp DNA fragment. Expression of the calponin h1 gene was determined by RT-PCR using the primers 5’-GTCTGTCATCTGACCTC-3’ (forward) and 5’-TCCGTCGCAGAATGCGC-3’ (reverse), which produced 1295-bp cDNAs of calponin h1 transcripts.

We compared various phenotypes between the mice homozygous null for the calponin h1 gene (CN−/−) and the mice carrying only the normal calponin h1 gene (CN+/−); these mice were generated by mating mice that were heterozygous null for the calponin h1 gene. In the present work, mice with five to seven back-crosses to the C57 BL/6J background were used. B16 mouse melanoma cells were transplantable to all of the animals used in the present study. All animal-handling procedures were in accordance with the ethics protocol approved by the University of Medicine.

Western Blot and Immunostaining. The synthetic COOH-terminal peptide, Leu491-Ala597, of the mouse calponin h1 protein was coupled to keyhole limpet hemocyanin with N-maleimidobenzoyl-N-hydroxysuccinimide ester. An antiserum against the peptide was generated in rabbits as reported previously (19). The antibody was used for the immunoblot analysis as well as for the immunostaining of mouse tissues. Mesothelial cells from the mouse mesentery were cultured to confluency in a 60-mm dish and lysed with sample buffer for SDS-PAGE analysis. The lysed samples from mice were electrophoresed and used for Western blot analysis with the above antibody. For the PCR, exons 4 and 5 of the calponin h1 gene (CN−/−) were amplified to examine the genotype. Using RT-PCR, we detected cDNA fragments (1295 bp) and the neo-containing fragment were identified as the CN−/− genotype; mice exhibiting the CN+/− genotype; mice exhibiting no expression of calponin h1 transcripts in the CN+/− mice. As for the CN+/− mice, the expression of calponin h1 protein was evaluated by injecting 1 × 10^6 viable cells in 0.5 ml of HBSS into the tail or femoral veins of the mice. The mice were euthanized 17–21 days later, and each organ was examined for the formation of metastatic tumor nodules.

To examine peritoneal dissemination, gently trypsinized B16-F10 melanoma cells were suspended in HBSS, and 2.5 × 10^6 viable cells/0.5 ml were injected into the peritoneal cavity. After 15 days, the mice inoculated with the tumor cells were euthanized and examined for the existence of ascites and the number of tumor nodules formed in the peritoneum and on the liver.

Statistical Analysis. Statistical analyses between the groups (CN+/− and CN−/− mice) in each experiment were performed by the Student t test or the Kaplan-Meier method. In all analyses, the differences were considered to be significant at P < 0.05.

RESULTS

Immunohistochemistry of Human Malignant Tumor Tissues. We stained various human malignant tumors with anti-αSMA and monoclonal antihuman calponin h1 antibodies. Fig. 1 shows representative images of the human malignant tumor tissues, such as uterus carcinoma, angiosarcoma, and prostate cancer. The blood vessels were detectable by immunohistochemical staining for αSMA, where calponin h1 expression was very weak (Fig. 1A, thick arrows) in the blood vessels of the malignant tumors compared with the surrounding normal tissues (Fig. 1A, top row, thin arrows). To allow easy evaluation and highlight the RBCs in the lumens, enlarged figures are shown in the second row of Fig. 1A. In both angiosarcoma (Fig. 1B) and prostate cancer (Fig. 1C), the blood vessels that were recognizable by staining with anti-αSMA were faintly stained with the anticalponin h1 antibody. We also observed the same phenomena in various human skin pigmented tumors. Malignant melanoma blood vessels showed weaker staining with the anticalponin h1 antibody than the normal tissues and benign tumors. According to our immunofluorescence observations, the staining intensity with anti-αSMA was also weaker in the blood vessels of the malignant tumor tissues than in the surrounding normal tissues (data not shown), which is consistent with previous reports (15–17).

CN−/− Mice. The genotypes of mice were confirmed by amplification of the genomic DNA using PCR. Mice that exhibited the neo fragment (218 bp) but lacked the exon 4–7 (EcoRV-Apal) fragment were identified as the CN−/− genotype. Conversely, mice that exhibited the expected exon 4–7 fragment (EcoRV-Apal; 297 bp) but no neo fragment were identified as the CN+/− genotype; mice exhibiting both the 218- and 297-bp fragments were identified as the CN+/+ genotype. Using RT-PCR, we detected cDNA fragments (1295 bp) derived from calponin h1 transcripts in the CN+/+ mice, but we found no expression of calponin h1 transcripts in the CN−/− mice (data not shown). There was no enhanced or ectopic expression of neutral and acidic calponin mRNAs (data not shown), which is consistent with our previous report (19). Immunostaining with antimouse calponin h1 polyclonal antibody showed calponin h1 expression in the blood vessels of the thigh and ocular fundus in the CN−/− mice, whereas calponin h1 expression was not detected in the CN+/− mice (Fig. 2). As for the CN−/− mice, the expression of calponin h1 protein was much the same as in the CN+/− mice, so that the various phenotypes were compared between the CN−/+ and CN−/− mice.

Morphological Fragility of Smooth Muscle Cells in the Uterus of CN−/− Mice. Because we expected that the suppression of calponin h1 would affect cell-to-cell and cell-to-extracellular matrix adhesiveness, we initially carried out morphological examination on the smooth muscle cells of the uterus in the CN−/− mice, using electron microscopy...
microscopy. As shown in Fig. 3, the smooth muscle cells of the uterus of the CN^-/- mice were morphologically round in structure, and the cellular adhesion was clearly decreased.

**Alteration of Blood Vessel Structure in CN^-/- Mice.** Electron microscopy of the femoral artery showed that the adhesion of the endothelial cells to the elastic fiber was weaker in CN^-/- than in CN^+/+ mice (Fig. 4A). In the coronary artery, fibrosis between the endothelial and smooth muscle cells was frequently observed (Fig. 4B). Bleeding from the blood vessels of the ocular fundus was often detected (Fig. 4C).

In the lung veins and venules, the diameters of the lumina appeared to be larger in the CN^-/- than in the CN^+/+ mice (Fig. 5, A and B); the same phenomenon was also observed in the blood vessels of the ocular fundus. These results led us speculate that the walls of such blood vessels could become thin in association with the enlargement of the diameter of the blood vessels; we then observed by electron microscopy that the walls of the pulmonary arterioles clearly became thinner in the CN^-/- mice compared with the CN^+/+ mice (Fig. 5C). When examined by scanning electron microscopy, the surfaces of the capillaries in the lungs of the CN^-/- mice appeared rough and fragile compared with capillaries in the CN^+/+ mice (Fig. 5D).

**Fluorescein Leakage from Blood Vessels of the Retina in CN^-/- Mice.** On the basis of the structural fragility of blood vessels in the CN^-/- mice, we examined the permeability of retinal blood vessels because leakage from these blood vessels is easy to assay. When fluorescein was injected i.v. into the mice, fluorescein leakage was greater in the CN^-/- than in the CN^+/+ mice. As shown in Fig. 6, the leakage of fluorescein, which is recognizable by the image at the rim of the vessels, was observed 30 s after the injection of fluorescein; the diffusion of fluorescence was greatly expanded in CN^-/- mice 180 s after the injection period, whereas there was comparatively little leakage detected in the CN^+/+ mice 300 s after the injection.

**Increase in Hematogenous Metastasis of B16 Melanoma in CN^-/- Mice.** When B16-F10 cells were administered i.v. to the mice, the metastatic tumor nodules detected were present mainly in the lungs. The metastatic nodules were observed microscopically at ×6.4 magnification. Colonies with diameters larger than ~100 µm, which would correspond to clinically relevant metastases, were selected for counting. The average number of metastatic nodules counted was 116 in the CN^-/- mice and 180 in the CN^+/+ mice, respectively. As shown in Fig. 7, A and B, the metastasis of B16-F10 cells to the lung, as indicated by the relative number of metastatic nodules counted from replicate experiments, was significantly greater in the CN^-/- mice than in the CN^+/+ mice (P < 0.05). When we inoculated B16-F1 cells i.v. into the mice, more metastatic nodules were formed in the lungs of the CN^-/- than the CN^+/+ mice (data not shown). In addition to the metastasis in the lungs, metastatic nodules were also observed in the

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**Fig. 1.** Immunostaining of human uterus carcinoma, angiosarcoma, and prostate carcinoma. Shown are serial sections of human uterus carcinoma (A), angiosarcoma (B), and prostate carcinoma (C). Left panels, H&E staining; middle panels, immunostaining with anti-α-SMA (magnification, ×100); right panels, immunostaining with monoclonal anti-human calponin h1 antibody (magnification, ×100). In all of the tumors, calponin h1 was weakly expressed in the blood vessels. In A, thin arrows in the top row show blood vessels in the normal areas outside the tumors. The bottom row of A shows enlarged images of blood vessels to highlight the red cells. Thick arrows in A–C show blood vessels inside the tumors. Bars: 250 µm in the top row of A; 100 µm in bottom row of A and in B and C.
liver, skin, and diaphragm as well as other organs of the female mice, although the number of nodules was low compared with the lung. The number and frequency of metastases in various tissues also tended to be increased in the CN/H11002/H11002 mice (Table 1); consequently, the survival period for the CN/H11002/H11002 mice was significantly shorter than that for the CN/H11001/H11001 mice (Fig. 7).

**Fragile Peritoneum in CN/H11546/H11546 Mice.**
We cultured mesothelial cells from the CN/H11001/H11001 and CN/H11002/H11002 mice and examined the expression of calponin h1 in the mesothelial cells by Western blot analysis with a specific polyclonal antibody against mouse calponin h1. Fig. 8 shows that calponin h1 was clearly expressed in the cultured mesothelial cells of the CN/H11001/H11001 but not the CN/H11002/H11002 mice.

We next examined the ultrastructure of the peritoneum. Fig. 9 shows that the mesothelial cells in the CN/H11002/H11002 mice had shrunken or retracted cytoplasm and that the subserosal connective tissue was thinner than in the CN/H11001/H11001 mice. Furthermore, the extracellular matrix of the peritoneum appeared to be disorganized in the CN/H11002/H11002 mice compared with the matrix in the CN/H11001/H11001 mice; an organized parallel arrangement of collagen fibers was seen in the CN/H11001/H11001 but not in the CN/H11002/H11002 mice.

**Enhancement in Peritoneal Carcinomatosa in CN/H11546/H11546 Mice.**
When we inoculated B16-F10 cells into the peritoneal cavities of the mice, the severity of peritonitis carcinomatosa was greater in the
than in the CN\(^{-/-}\) mice; a representative result is shown in Fig. 10. Similarly, the quantity of ascites and number of tumor nodules on the peritoneum and on the liver were much greater in the CN\(^{-/-}\) mice (Table 2).

DISCUSSION

In this study, we used immunohistochemical techniques to detect low expression of calponin h1 in the blood vessels of malignant human tumor tissues and discovered a fragile structure of the blood vessels and peritoneum in the CN\(^{-/-}\) mice, which possibly leads to an increase in hematogenous metastasis and peritoneal dissemination of cancer cells. The results in the CN\(^{-/-}\) mice together with the phenomena found in the human tumor tissues indicate that the suppression of calponin h1 in the blood vessels or peritoneum is an important factor for increasing cancer metastasis.

Although there are a number of factors that determine metastasis of cancer cells, the results in the present study suggest that the integrity of the blood vessels and peritoneum of the host is an equally important factor. The process by which cancer cells intra- and/or extravasate through vessels and the peritoneum must be one of the rate-limiting steps of metastasis that depends on whether cells constituting vessels and the peritoneum retract or not. Such retraction could occur as a result of the disruption of intercellular adhesion and/or cellular attachment to the extracellular matrix. The mechanisms of cellular adhesion, such as the adherence junction and the tight junction, are connected and regulated by actin filaments. Thus, the reduction in cellular adhesion is probably induced by the fragility of the actin filaments as a result of the suppression of stabilizers, such as calponin, as shown in the present study, as well as the down-regulation or mutation of adhesion molecules in the cellular plasma membrane.

In the blood vessels, calponin h1 is detected mainly in the smooth muscle cells and pericytes; our present study thus provides information on the vessels containing smooth muscle cells and pericytes, such as arteries/arterioles and veins/venules. If, as is the general belief, metastatic cells rarely intra- and extravasate through arteries/arterioles, the enhanced hematogenous metastasis, especially pulmonary metastasis, of B16-F10 cells in the CN\(^{-/-}\) mice (Fig. 7 and Table 1) seemingly can be attributed to the fragility of veins/venules induced by the deletion of calponin h1. Actually, the
HIGHLY FREQUENT METASTASES IN CALPONIN h1-DEFICIENT MICE

metastatic tumor nodules on the lungs of CN/H11002/H11021/P/H11003/survival rates of mice receiving i.v. injections of B16-F10 cells (5

an image for the venules as for the arterioles. In contrast, it has

been reported that calponin h1 can be detected in the endothelial cells by RT-PCR (11); we therefore thought that suppression of calponin h1 in the endothelial cells as well as in the vascular smooth muscle cells leads to structural immaturity and weakness in

Table 1 Extrapulmonary metastases

In these experiments, 5 × 10⁵ cells/mouse were injected i.v. into female mice. In experiment 1, metastases were examined 21 days after the injection with tumor cells. In experiment 2, survival rate of the animals was assessed, so that extrapulmonary metastases were examined when the mice died mainly of lung metastases. Extrapulmonary metastases were not detected in the male mice.

<table>
<thead>
<tr>
<th>Organ (no. of metastatic nodules)</th>
<th>CN⁺/+</th>
<th>CN⁻/⁻</th>
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<tr>
<td>Kidney (2); skin (1); ovary (1)</td>
<td></td>
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<tr>
<td>diaphragm (1); stomach (1)</td>
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<td>n = 7</td>
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<tr>
<td>Kidney (2); diaphragm (3); uterus (1); thorax (1)</td>
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<td>n = 9</td>
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Fig. 7. Lung metastasis of i.v. injected B16-F10 cells (5 × 10⁵ cells/0.5 ml/mouse). The data in A reflect a composite from triplicate experiments. We used mice back-crossed 6–7 times for these experiments. The average number of lung metastases of the CN⁺/+ mice group in each experiment was arbitrarily adjusted to 100, and the relative number of lung metastases of each mouse used in the experiment was calculated. This relative number corresponding to the lung metastases was used for the statistical comparison between the CN⁺/+ and CN⁻/⁻ groups. A, the relative average number of tumor nodules of B16-F10 cells in the lung was significantly larger (P < 0.05) in the CN⁻/⁻ mice (152.0 ± 15.2; n = 21) than in the CN⁺/+ mice (100.0 ± 8.6; n = 24). Bars, SE. B, metastatic tumor nodules on the lungs of CN⁻/⁻ (bottom) and CN⁺/+ (top) mice. C, survival rates of mice receiving i.v. injections of B16-F10 cells (5 × 10⁵ cells/mouse). In the experiment of panel C and Table 1, female mice were used. Dashed line, CN⁻/⁻ mice (n = 9); solid line, CN⁺/+ mice (n = 9). The survival period of the CN⁻/⁻ mice (22.9 ± 0.3 days) was significantly shorter than that of the CN⁺/+ mice (24.9 ± 0.6 days; P < 0.05).

diameters of the pulmonary veins and venules in the CN⁻/⁻ mice were enlarged, and the walls of the arterioles were thinner in the CN⁻/⁻ than in the CN⁺/+ mice (Fig. 5), indicating the fragility of the blood vessels in the CN⁻/⁻ mice. We believe that the walls of the venules in the CN⁻/⁻ mice should be thinner compared with the CN⁺/+ mice, although it was technically difficult to show as clear an image for the venules as for the arterioles. In contrast, it has

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Fig. 8. Western blot analysis of the cultured mesothelial cells with rabbit antimouse calponin h1 polyclonal antibody. Mesothelial cells were prepared and cultured according to the method of Akedo et al. (26). Calponin h1 (molecular mass, 34 kDa) was detected in the cells from the CN⁺/+ but not the CN⁻/⁻ mice.

Fig. 9. Electron microscope image of the peritoneum. The mesothelial cells (indicated by straight lines) were retracted in the peritoneum of the CN⁻/⁻ mice compared with those of the CN⁺/+ mice. Collagen fibers of the peritoneum were thinner in the CN⁻/⁻ mice. The thickness of the peritoneum shown is 28 μm in the CN⁺/+ and 17 μm in the CN⁻/⁻ mouse. Bar, 10 μm.

Fig. 10. Peritoneal dissemination of B16-F10 cells inoculated at 2.5 × 10⁵ cells/0.5 ml/mouse. More widespread dissemination of B16-F10 cells was observed in the CN⁻/⁻ mice compared with the CN⁺/+ mice.

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all types of blood vessels, including capillaries. Because both the pulmonary arterioles and venules in CN\(^{-/-}\) mice were morphologically different from those in the CN\(^{+/+}\) mice, the capillaries existing between them should be somehow changed in the CN\(^{-/-}\) mice. As expected, when observed with scanning electron microscopy, the surfaces of capillaries in the lungs in the CN\(^{-/-}\) mice appeared rough and irregular compared with those in the CN\(^{+/+}\) mice (Fig. 5D). These morphological changes in the endothelial cells suggested to us the fragility of the capillaries, although direct verification remains to be done.

How calponin h1 is suppressed in human tissues is an important issue, although the mechanism of gene regulation has been only partially clarified. The 5\(^{\prime}\) flanking region of the human calponin h1 gene has been shown to have a cis-acting domain for interaction with a methylated DNA-binding transcription repressor (20). Moreover, the expression of smooth muscle cell-specific molecular markers is reportedly affected by growth factors, such as PDGF (17, 21, 22) and the expression of smooth muscle cell-specific molecular markers is indicative of the immaturity and fragile structure of the blood vessels and serves as a useful molecular marker for the differential diagnosis of malignant from benign tumors. Finally, we believe that there might be a good possibility for gene therapy with calponin h1, not only to suppress the growth of tumor cells but also to strengthen the physical barriers of the host system against tumor formation.

**ACKNOWLEDGMENTS**

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**REFERENCES**


Announcements

MEETING OF THE RADIATION RESEARCH SOCIETY

The annual meeting of the Radiation Research Society will be held at the State University of Iowa, Iowa City, on June 22-24, 1953. The Society will be the guest of the University, and all meetings will be held on the campus. The program will consist of: (1) Two symposia, one on "The Effects of Radiation on Aqueous Solutions," which includes the following speakers: E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen. The second is "Physical Measurements for Radiobiology" and companion talks by Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne S. Harris. (2) On Monday night, June 22, a lecture by Dr. L. W. Alvarez on meson physics has been tentatively scheduled. On Tuesday night, June 23, Dr. L. H. Gray of the Hammersmith Hospital, London, will speak on a topic to be announced. Dr. Gray's lecture is sponsored by the Iowa Branch of the American Cancer Society. Those desiring to report original research in radiation effects, or interested in attending or desiring additional information, please contact the Secretary of the Society, Dr. A. Edelmann, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

ERRATUM

The following correction should be made in the article by Beck and Valentine, "The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia. I. Glycolysis and Respiration," November, 1952, page 891; substitute for the last paragraph:

The data in Table 8 permit several interesting calculations. If one compares the amount of glucose actually disappearing with the sum of the amount equivalent to lactic acid produced plus that equivalent to O2 consumption, it is seen that the amount of glucose "cleavage products" exceeds the amount of glucose utilized by 12 per cent in N and 27 per cent in CML and is exceeded by the glucose utilized by 16 per cent in CLL. If the assumption is made that, in this respect, the myeloid and lymphoid cells of leukemia are similar to those of normal blood, it may be that the computed normal figure represents a summation of the myeloid (M) and lymphoid (L) cells that make up the normal leukocyte population. Thus, if $M = +0.27$ and $L = -0.16$ and the normal differential is 65 per cent M and 35 per cent L, then

$$0.65 \times (+0.27) + 0.35 \times (-0.16) = +0.12,$$

a figure identical to the observed $+0.12$ for normal leukocytes.
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