Symposium on the Diagnosis of Cancer*

Malignolipin

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In examining the affinity of various cell components for protoporphyrin, it was noticed that small bodies with a very marked affinity for protoporphyrin existed in cancer tissues, mainly extracellularly. Attempts were made to isolate from human cancer tissues the substance which constitutes these small bodies and which has an affinity for protoporphyrin. During the isolation process, particular care was taken to exclude sphingomyelin, the substance with the affinity for protoporphyrin in normal cells. A new phospholipid, malignolipin, was thus obtained in pure crystalline state by solvent fractionation. This malignolipin was found to be composed of a fatty acid, spermine, phosphoric acid and choline. As malignolipin appeared to be found only in cancer tissues, or in the blood or in the ascites of cancer-bearing subjects, but never in normal subjects, it was stated in 1958 that the detection of malignolipin in blood would serve for the diagnosis of cancer. Since then the cancer specificity of malignolipin has been examined from various standpoints. The outline of the results will be presented here, together with the results of the investigation concerning the methods for the detection.

I. On the cancer specificity of malignolipin

1. Examination with the histological detection of malignolipin based on its affinity for protoporphyrin

Malignolipin can never be detected in normal human tissues (liver, kidneys, alimentary tract, lungs, heart, pancreas, spleen, urinary bladder, prostate, ovaries and testicles) nor in non-cancerous

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tissues (hepatitis, liver cirrhosis, stomach ulcer, renal tuberculosis, prostate hypertrophy, human embryos of 2-3 months, and chicken embryos of various stage), but can always be detected in cancerous tissues (an esophageal cancer, 13 stomach cancers, a cancer of cecum, 3 rectal cancers, an uterine cancer, a renal adenocarcinoma, a Gra-witz's tumor, a breast cancer, 2 urethral cancers, 2 seminomas, 5 cancers of urinary bladder, a cancer of prostate, a malignant lymphogranuloma, and a reticulosarcoma), as far as has been examined.

2. Examination with the paper-chromatographic detection of malignolipin in blood

The results obtained with blood of human subjects are shown in the table. Out of 1502 cases, which yielded decisive results, the blood of 490 patients bearing histologically proved malignant tumor showed positive malignolipin test without exception, and the blood of 524 subjects highly exclusive of malignancy, including 95 normal subjects and 40 pregnant women, showed negative results without exception. After a complete removal of malignant tumors, the blood malignolipin test turned out negative within a short period of time (shortest 16 days as examined), but remained positive after an incomplete removal. In negative cases, the test remained negative even after the X-ray irradiation. In some positive cases, the test turned negative after a short or long period of irradiation, and the prognosis of such cases was good. In some cases, it turned negative after the irradiation, but returned to positive at the end of a 7 or 11 month period after the irradiation. In some cases, it remained positive even after a long period of irradiation. The prognosis of such cases was not good. There were 10 cases which had been clinically diagnosed as bearing an malignant tumor, although the blood malignolipin test was negative, but later, on operation or specific therapy, these were ascertained to have no malignant tumor. On the other hand, there were 22 cases in which the blood malignolipin test was positive although no clinical sign suggesting the existence of a malignant tumor could be found. However, by careful histological examination or follow-up observations, these cases were found later to have a malignant tumor. Among these cases, 9 were ascertained to have a malignant tumor in so early a stage of development that it could not be discerned, even on operation, to be a malig-

Blood malignolipin test (paper-chromatographic) in 1646 human subjects
Results of the total cases practised in the present laboratory from Sept. 1st, 1957 to Feb. 28th, 1962

Final diagnosis	Number of positive cases			Number of negative cases			Number of cases remained to be failed		
	Successful on the 1st trial	Successful on the 2nd trial*	Sum	Successful on the 1st trial	Successful on the 2nd trial*	Sum	Failed on the 1st trial**	Failed on the 2nd trial	Sum
1. Histologically proved malignant tumor	465	25	490	0	0	0	34	0	34
a. Proved before the test	89	6	95	0	0	0	10	0	10
b. Proved after the test	193	6	199	0	0	0	18	0	18
c. Transferred from 3c	20	2	22	0	0	0	0	0	0
d. After partial resection (within a year)	122	6	128	0	0	0	4	0	4
e. „ (more than a year)	41	5	46	0	0	0	2	0	2
2. Malignant tumor doubted	233	15	248	56	5	61	31	0	31
a. Going to be ascertained	105	4	109	24	2	26	12	0	12
b. Not ascertained	128	11	139	32	3	35	19	0	19
3. Diseases other than malignant tumor	145	6	151	398	19	417	72	0	72
a. Histologically proved	0	0	0	36	4	40	6	0	6
b. No evidence of malignancy	0	0	0	263	13	276	40	0	40
c. Not yet certain	145	6	151	27	1	28	22	0	22
d. After total resection (within a year)	0	0	0	52	1	53	1	0	1
e. „ (more than a year)	0	0	0	20	0	20	3	0	3
4. Pregnancy (including lately confined)	0	0	0	40	0	40	3	0	3
5. Normal	0	0	0	92	3	95	4	0	4

* Failed on the 1st trial, but successful on the 2nd trial.

** Failed on the 1st trial, and the 2nd trial could not be carried out.

1. includes 37 kinds of cancer, and 9 kinds of sarcoma.

3a and 3b include 87 kinds of diseases, especially precancerous diseases and benign tumors.

4. includes every month's one.

nant tumor. Among these 9 cases, 3 were cases of stomach cancer confined to mucosa, 4 were cases of breast cancer, one was a case of Grawitz's tumor, and one was a case of cancer of the gallbladder.

Malignolipin cannot be detected in the blood of normal mice (29 cases) or rats (14 cases), but can always be detected in the blood of rats (41 cases) and mice (57 cases) bearing fully grown malignant tumors, and also in the tumor ascitic fluid of rats (13 cases) and mice (352 cases), irrespective of kinds of tumor and animal species. On examining the blood of 312 mice inoculated with Bashford's carcinoma 63, NF sarcoma, Crocker's sarcoma 180 or Ehrlich's ascites cancer (solid and ascitic), one finds that malignolipin begins to appear in the blood as early as 1-3 days after the inoculation of malignant tumors, and becomes detectable in the blood of all cases by the end of 1-6 days after the inoculation; by the end of 24 hrs. after the inoculation in case of an ascites tumor and by the end of 4-6 days after the inoculation in case of a solid tumor.

3. Detection of malignolipin in blood and tissues by the picrate method

The principle of this method is to isolate malignolipin from blood or tissues as its picrate, and to detect its presence or to measure its amount by means of the optical property of its ethanol solution. As for the detection of malignolipin in the blood of human subjects, this method has been used since the middle of 1960 together with the preceding paperchromatographic method, and in every case the same results have been obtained. Malignolipin cannot be detected with this method in the following materials: whole body of 3 normal rats and 16 normal mice; in kidneys, liver, spleen, testicles and intestinal tracts of 10 normal mice; in whole blood, kidneys, liver, stomach, intestine, skeletal muscle, cerebrum, cerebellum, heart, spleen and suprarenals of 2 normal guinea pigs and 4 normal rabbits; in whole blood of a normal sheep (examined 7 times with samples of 1-5 ml. drawn at various intervals); in the body other than embryo of 2 pregnant mice; in 16 embryos of normal mice; in the regenerating liver of 5 normal rats after the partial hepatectomy. Malignolipin can always be found when this method is used on various malignant tumors, the malignolipin content being about 25 mg. per 100 g. wet weight or 100 ml. After the intraperitoneal inoculation

of Ehrlich's ascites cancer, malignolipin appears in various tissues as early as at the end of a 24 hour period after inoculation, and with the lapse of time, increases gradually in the blood and in the entire body.

4. An antigenic property of malignolipin

To examine the antigenic property of malignolipin, the substance in picrate form was isolated from various kinds of malignant tumors. While free malignolipin is very hygroscopic and labile, its picrate is stable and is not hygroscopic. Before malignolipin is used, picric acid must be removed from the malignolipin picrate, and the ammonium salt of malignolipin in pure crystals obtained. The ammonium salt of malignolipin is not hygroscopic, and readily dissolves in a physiological saline solution. Samples from the different kinds of tumors yield the same findings, as to the picric acid content as well as the melting point of malignolipin picrate, the shape of crystal, the solubilities and the optical properties of both malignolipin picrate and its ammonium salt.

The subcutaneous injection of malignolipin (ammonium salt) into normal rats, mice and rabbits results in the production of a complement-combining specific antibody in those animals. Anti-malignolipin sera cause no precipitation with malignolipin, but they combine complement in the presence of malignolipin. Complement fixation cannot be exhibited either in the absence of malignolipin or antimalignolipin sera, or with normal sera and malignolipin. Anti-malignolipin sera does not fix complement in the presence of Wassermann's antigen or Kahn's antigen. The complement combining antibody can be removed from anti-malignolipin sera by adsorption to Ehrlich's cancer cells or Yoshida sarcoma cells, while the complement fixation titer of the anti-malignolipin sera shows no reduction after the treatment with the blood corpuscles of normal sheep or normal rats, or with acetone-dried powder of normal rat liver, kidney or spleen. Samples of malignolipin taken from different kinds of tumors show no difference in their antigenic property to each other.

5. Combination of fluorescent anti-malignolipin sera

The fluorescent anti-malignolipin sera (of rats and rabbits) after the absorption with normal liver combine nowhere with the smears

of normal rat serum, egg white or normal blood (human, rats and mice). They combine everywhere with smears made of normal rat serum or egg white which have been mixed with malignolipin, and also with smears of Ehrlich's cancer ascites, Yoshida sarcoma ascites, or blood of cancer bearing mice and rats, the combination being more marked in the fluid part than the cellular part. Sections of NF sarcoma combine markedly with the fluorescent anti-malignolipin sera, but sections of the liver of normal rats and mice do not combine. When sections of NF sarcoma has been pre-incubated with non fluorescent antisera, no area of the sections cannot combine with the fluorescent antisera. The fluorescent normal sera, after they have been absorbed by normal liver, combine with no area of the above mentioned smears and sections.

From the above results it is indicated (1) that malignolipin is specific to malignant tumors, (2) that it is common to malignant tumors, and (3) that it appears in the blood of subjects bearing a malignant tumor from the early stage of its development. Accordingly, the detection of malignolipin in the blood may be useful for the exact diagnosis, especially for the early diagnosis, of malignant tumors.

II. On the practical method for the detection of malignolipin in the blood

As for the methods of detecting malignolipin in the blood, the following three are available at the present; the paper-chromatographic method, the picrate method and the histological method by means of fluorescent antisera. The last method is not practical, however, because in order to obtain a safe smear, the period of time between the drawing of the blood and the final observation must be sharply limited. The former methods yield good results, if they are carried out strictly and carefully. The paper-chromatographic method fails in 217 out of 1,646 cases, that is 13.2%, on the first trial. But if the test is repeated, and if it is made very carefully, with adequate time being taken to carry out the exact procedure, it is successful in all cases. The picrate method is always successful, but the procedure is more complicated than is that of the paper-chromatographic method. The authors are now on the way to devise a more simple, but exact method for the detection of malignolipin. This will be reported in the near future.