Correction

MEDICAL SCIENCES. For the article "Genes overexpressed in different human solid cancers exhibit different tissue-specific expression profiles," by Jacob Bock-Axelsen, Joseph Lotem, Leo Sachs, and Eytan Domany, which appeared in issue 32, August 7, 2007, of *Proc Natl Acad Sci USA* (104:13122–13127; first published July 30, 2007; 10.1073/pnas.0705824104), the authors note that the author name Jacob Bock-Axelsen should have appeared as Jacob Bock Axelsen. The online version has been corrected, and the corrected author line appears below.

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www.pnas.org/cgi/doi/10.1073/pnas.0707795104

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Genes overexpressed in different human solid cancers exhibit different tissue-specific expression profiles

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Contributed by Leo Sachs, June 21, 2007 (sent for review May 20, 2007)

We have analyzed gene expression in different normal human tissues and different types of solid cancers derived from these tissues. The cancers analyzed include brain (astrocytoma and glioblastoma), breast, colon, endometrium, kidney, liver, lung, ovary, prostate, skin, and thyroid cancers. Comparing gene expression in each normal tissue to 12 other normal tissues, we identified 4,917 tissue-selective genes that were selectively expressed in different normal tissues. We also identified 2,929 genes that are overexpressed at least 4-fold in the cancers compared with the normal tissue from which these cancers were derived. The overlap between these two gene groups identified 1,340 tissue-selective genes that are overexpressed in cancers. Different types of cancers, including different brain cancers arising from the same lineage, showed differences in the tissue-selective genes they overexpressed. Melanomas overexpressed the highest number of brain-selective genes and this may contribute to melanoma metastasis to the brain. Of all of the genes with tissue-selective expression, those selectively expressed in testis showed the highest frequency of genes that are overexpressed in at least two types of cancer. However, colon and prostate cancers did not overexpress any testis-selective gene. Nearly all of the genes with tissue-selective expression that are overexpressed in cancers showed selective expression in tissues different from the cancers' tissue of origin. Cancers aberrantly expressing such genes may acquire phenotypic alterations that contribute to cancer cell viability, growth, and metastasis.

DNA microarray | human cancers | normal human tissues | tissue-selective gene expression

The genetic and epigenetic changes that lead to cancer development are associated with aberrant gene expression including overexpression of genes compared with the normal tissue from which the cancers originated (reviewed in ref. 1). Some of the overexpressed genes in many types of human cancers are normally expressed only in germ-line cells and are called cancer/testis genes (2–5). But not only testis genes are overexpressed in cancer. Using DNA microarray and cluster analysis of gene-expression data, we have previously shown that some of the genes that are highly expressed in mouse and human leukemia cell lines and in leukemic cells from patients were preferentially expressed in various normal tissues including testis, brain, kidney, lung, liver, and others (6, 7). These results indicated that leukemias express different genes whose normal expression profile shows selectivity for various nonhematopoietic tissues. Similar analysis of highly expressed genes in the human SW480 adenocarcinoma cell line has suggested that solid cancers might also possess the ability to overexpress genes whose normal expression profile shows selectivity for various normal tissues that are different from the tissue in which the cancer originated (7). We term the genes that show a tissue-selective expression profile in different normal tissues "tissue-selective genes." To determine to what extent this phenomenon is general to different types of human solid cancers, we have now analyzed DNA microarray data obtained from 566 samples of 13 different normal human tissues and from 1,401 samples of 12 different types of solid cancers that originated from these tissues. The results indicate that nearly all of the tissue-selective genes overexpressed in the cancers showed selective expression in tissues that are different from the tissue in which the cancers originated, that there were differences in the tissue-selective genes overexpressed in different types of solid cancers, and that $<0.3\%$ of the overexpressed genes in the cancers were selectively expressed in the tissue from which the cancer originated.

Results

Determination of Tissue-Selective Gene Expression in Normal Human Tissues. Using 14 DNA microarray data sets including 566 samples obtained from 13 normal human tissues including blood, brain, breast, colon, endometrium, kidney, liver, lung, skin, ovary, prostate, testis, and thyroid [supporting information (SI) Table 4], we have analyzed tissue-selective gene expression. Tissue-selective gene expression was determined based on a statistically significant difference in expression with management of false discovery rate at 5% and at least a 4-fold higher expression value of the mean for each gene in a given normal tissue compared with all other normal tissues, as described in *Materials and Methods*. Using these criteria we found that of the 21,700 probe sets (PSs) that were common to all of the data sets included in our study, 4,917 PSs (22.6%) showed a tissue-selective expression. The numbers of PSs with selective expression in different normal tissues are shown in Table 1 and indicate that only a small number of PSs assigned to a particular tissue-selective group overlapped with another tissue-selective group. Of the 4,917 tissue-selective PSs, 4,462 PSs (90.7%) showed selective expression in just one normal tissue type, 420 PSs (8.5%) in two normal tissue types, 34 PSs (0.7%) in three normal tissue types, and only 1 PS in four different normal tissues. The complete list of genes assigned to the different tissue-selective groups is shown in SI Table 5. Several examples of the relative expression profile of the PSs included in the different tissue-selective groups are shown in Fig. 1. The low degree of overlap between genes that were assigned to different tissue-selective groups indicates that our method of identifying normal tissue-selective gene expression is appropriate and can be used in the analysis of gene expression in cancers derived from these tissues.

Determination of Overexpressed Genes in Different Human Solid Cancers. In our previous analysis of gene expression in leukemia cells (6, 7), we selected only those genes that were highly expressed in the leukemias, namely genes whose expression levels were above the 85th percentile. Genes that were highly expressed in leukemia but were not highly expressed in normal

Author contributions: J.B.A., J.L., L.S., and E.D. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

Abbreviation: PS, probe set.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0705824104/DC1.

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Table 1. The overlap between different tissue-selective PS in normal human tissues

Normal tissue	Blood	Brain	Breast	Colon	Endomet	Kidney	Liver	Lung	Ovary	Prostate	Skin	Testis	Thyroid
Blood	**(624)**	0	1	0	6	0	2	7	1	25	3	2	14
Brain	0	**(634)**	2	0	0	0	3	0	4	2	2	1	2
Breast	1	2	**(313)**	7	15	3	10	36	9	29	49	3	7
Colon	0	0	7	**(244)**	0	0	9	2	0	3	4	5	2
Endomet.	6	0	15	0	**(162)**	2	3	4	19	7	11	0	5
Kidney	0	0	3	0	2	**(449)**	10	0	2	1	1	1	5
Liver	2	3	10	9	3	10	**(578)**	14	4	10	7	0	4
Lung	7	0	36	2	4	0	14	**(878)**	7	36	7	0	34
Ovary	1	4	9	0	19	2	4	7	**(106)**	4	9	5	5
Prostate	25	2	29	3	7	1	10	36	4	**(514)**	30	5	21
Skin	3	2	49	4	11	1	7	7	9	30	**(210)**	0	6
Testis	2	1	3	5	0	1	0	0	5	5	0	**(272)**	2
Thyroid	14	2	7	2	5	5	4	34	5	21	6	2	**(424)**

Values shown in bold in parentheses are the number of PSs showing at least a 4-fold higher expression in one tissue compared with all other tissues, called tissue-selective gene groups. Other values are the overlap of tissue-selective genes between the different tissue-selective groups in the matrix. Endomet, endometrium.

blood cells were scored as overexpressed genes. This analysis was thus restricted only to those genes that are both highly expressed in human leukemia and are overexpressed compared with normal blood. Because genes that are not highly expressed could still be overexpressed in cancer compared with the tissue from which the cancer originated, we have now used nine DNA microarray data sets, including 1,401 samples of 12 different types of human solid cancers (SI Table 4) to identify all of the genes that are overexpressed in each cancer type compared with its normal tissue of origin. Using the same criteria described above for the normal tissues, we found 2,929 PSs that are overexpressed in the different types of cancer compared with the tissue from which these cancers were derived (see complete list of overexpressed genes in different cancers in SI Table 6). The results indicate that melanoma overexpressed the highest (728 PSs) and thyroid cancer the lowest (35 PSs) number of genes (Fig. 2 and Table 2). Nine of the 12 types of cancer examined in our analysis had >100 overexpressed PSs (Fig. 2 and Table 2).

Identification of Tissue-Selective Genes Overexpressed in Different Human Solid Cancers. As previously shown, mouse and human leukemias overexpress genes that are selectively expressed in various normal nonhematopoietic tissues (6, 7), and the colorectal carcinoma cell line SW480 also overexpressed genes that are selectively expressed in normal tissues other than the tissue of origin (7). We have now compared in different types of human solid cancers the frequency of overexpressed genes that show such tissue-selective expression profiles. Analysis of the overlap between the 2,929 PSs that are over expressed in the 12 cancer types tested and the 4,917 PSs that show tissue-selective expression in different normal tissues identified 1,340 tissue-selective PSs that were overexpressed in these cancers. Of these 1,340 PSs, 1,087 PSs (81.1%) showed selective expression in one normal tissue, 224 PSs (16.7%) in two tissues, 28 PSs (2.1%) in three tissues and 1 PS in four tissues (for the complete list, see SI Table 7). The results indicate that ≈45% of the genes that are overexpressed in cancers (1,340 of 2,930 PSs) have a tissue-selective expression profile in normal human tissues, whereas the remaining ≈55% of the cancer overexpressed genes did not show such a tissue-selective expression in the normal tissues we analyzed.

Not all cancer types overexpressed the same tissue-selective genes (SI Table 8). Among the tissue-selective genes that are overexpressed in the different cancers, testis-selective genes showed the highest (61%) and brain-selective genes showed the lowest (14%) frequency of overexpression in two or more cancer

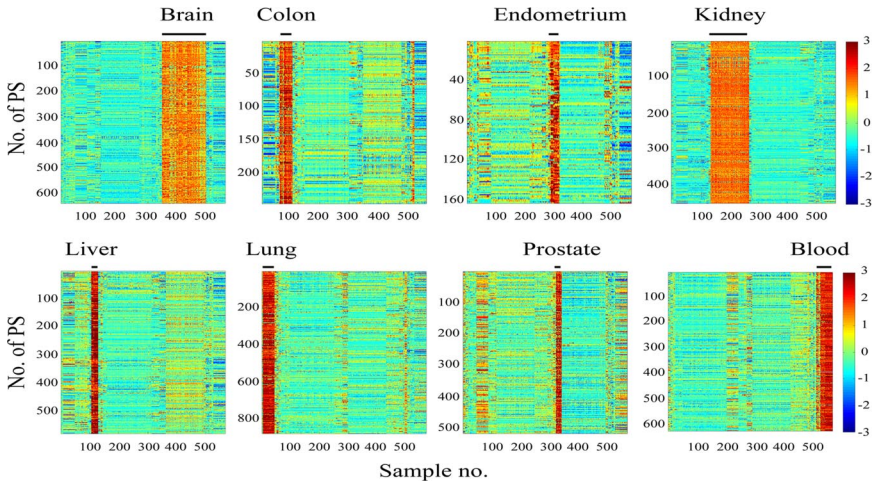

Fig. 1. Examples of the expression profile of tissue-selective PSs. The expression level of each PS in all samples of all normal tissues was determined. Those PSs whose expression levels in a given tissue were at least 4-fold higher than in all other normal tissues were scored as a tissue-selective PSs as described in *Materials and Methods*. The bars above the panels indicate the sample numbers of the indicated normal tissues.

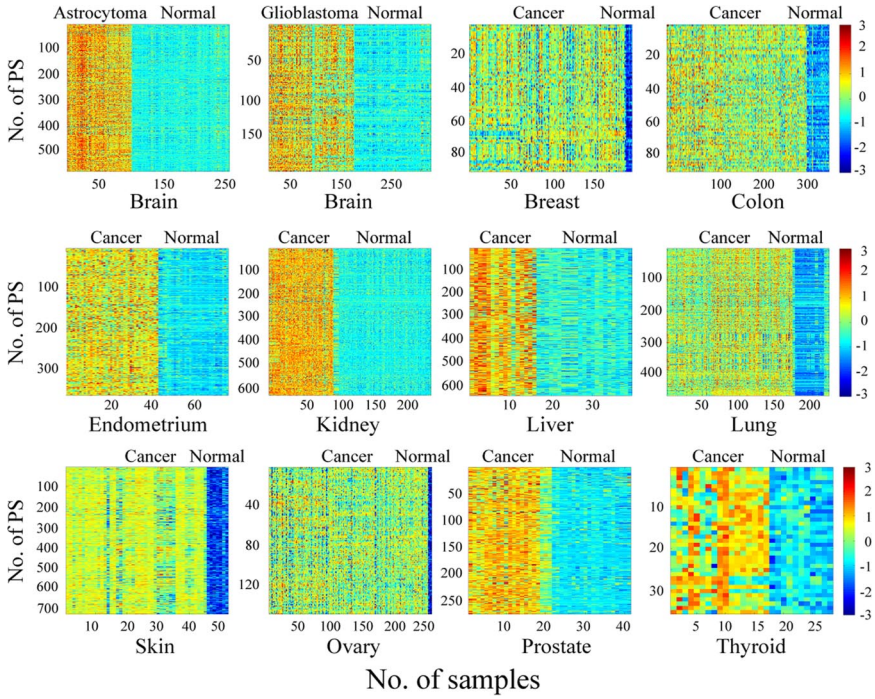

Fig. 2. Overexpressed PSs in different human solid cancers. The expression level of all PSs in all samples of different types of human solid cancers were compared with the expression level in all samples of the normal tissues in which these cancers originated. Those PSs whose expression level in a given type of cancer was at least 4-fold higher than in the corresponding normal tissue were scored as overexpressed PSs as described in *Materials and Methods*.

types (Table 3). Testis-selective genes showed the highest (29%) and brain-selective genes showed the lowest (0%) frequency of overexpression in at least four types of cancer (Table 3). Of all the other tissue-selective genes, the frequency of those that are overexpressed in at least two cancer types ranged from 25% to 46%, and those overexpressed in at least four cancer types ranged from 2.7% to 12.5% (Table 3). Only six tissue-selective genes, one testis-selective gene, two colon-selective genes, one prostate-selective gene, one endometrium-selective gene, and one gene selective for endometrium, ovary, and skin were overexpressed in at least 7 of the 12 cancer types tested (SI Table 8).

Analysis of each cancer type indicated that all types of cancer overexpressed genes that are selectively expressed in normal tissues different from the tissue in which the cancer originated (Table 2 and SI Table 8). Of all overexpressed genes in cancers, colon cancer showed the highest (81%) and melanoma showed the lowest (17%) frequency of genes with noncolon and nonskin tissue-selective expression, respectively. In all other cancer types, the frequency of overexpressed genes whose tissue selectivity was different from the tissue in which the cancers originated ranged from 40% to 65%. Melanoma overexpressed the highest number (45 PSs) of brain-selective genes compared with all other cancer types (Table 2). As with the colorectal carcinoma cell line SW480 (7), colon cancers and prostate cancers did not overexpress any testis-selective genes (Table 2).

We showed (7) that the number of nonhematopoietic genes

Table 2. Number of tissue-selective PSs that are overexpressed in human cancers

Overexpressed PSs		No. of tissue-selective PSs												
Cancers	No.	Blood	Brain	Breast	Colon	Endomet	Kidney	Liver	Lung	Ovary	Prostate	Skin	Testis	Thyroid
Brain														
Astrocytoma	575	42	**(5)**	22	8	20	10	31	89	13	87	15	23	32
Glioblastoma	195	18	**(6)**	6	6	19	4	18	26	8	28	10	8	7
Breast	90	4	0	**(0)**	6	7	3	4	1	2	2	0	17	2
Colon	89	6	2	17	**(1)**	20	3	6	14	3	30	9	0	3
Endomet.	361	17	16	26	15	**(0)**	13	19	91	4	46	13	11	22
Kidney	633	69	26	25	12	30	**(2)**	52	134	7	73	19	10	39
Liver	637	34	20	48	48	47	16	**(0)**	71	23	81	36	19	25
Lung	465	9	13	40	55	20	20	28	**(0)**	6	38	50	21	14
Ovary	149	3	0	6	13	5	7	1	7	**(0)**	5	4	16	6
Prostate	275	12	7	12	13	3	10	14	46	2	**(2)**	5	0	9
Skin														
Melanoma	728	17	45	14	2	9	7	10	31	5	5	**(3)**	11	7
Thyroid	35	2	5	2	0	1	0	6	6	1	2	2	1	**(0)**

Values shown in bold in parentheses are the numbers of cancer-overexpressed PSs whose expression profile in normal tissues is selective for the tissue in which the cancers originated. Other values are the number of cancer-overexpressed PSs whose expression profile in normal tissues is selective for tissues that are different from the tissue in which the cancers originated. Endomet, endometrium.

Table 3. The frequency of tissue-selective PSs overexpressed in more than one type of cancer

Normal tissue selectivity	No. of tissue-selective PSs overexpressed in cancers			
	Total	In only one type of cancer	In at least two types of cancer	In at least four types of cancer
Blood	135	81 (60.0)	54 (40.0)	9 (6.7)
Brain	122	105 (86.1)	17 (13.9)	0 (0)
Breast	123	66 (53.7)	57 (46.3)	7 (5.7)
Colon	117	88 (75.2)	29 (24.8)	8 (6.8)
Endometrium	91	45 (49.5)	46 (50.5)	9 (9.9)
Kidney	59	36 (61.0)	23 (39.0)	2 (3.4)
Liver	121	72 (67.8)	39 (32.2)	6 (5.0)
Lung	323	196 (60.7)	127 (39.3)	12 (3.7)
Ovary	40	23 (57.5)	17 (42.5)	5 (12.5)
Prostate	234	130 (55.6)	104 (44.4)	19 (8.1)
Skin	97	61 (62.9)	36 (37.1)	7 (7.2)
Testis	51	20 (39.2)	31 (60.8)	15 (29.4)
Thyroid	110	71 (64.5)	39 (35.5)	3 (2.7)

Values in parentheses are the percentage of total.

that are overexpressed in human acute lymphoid leukemias was 2.5-fold higher than in acute myeloid leukemias. We have now determined whether a difference could be detected between two different solid cancer types in the brain. Astrocytoma and glioblastoma are both brain cancers of astrocytic origin graded according to histological features and degree of malignancy as World Health Organization grades I-III and grade IV, respectively (8–10). The number of overexpressed genes in astrocytoma was almost 3-fold higher than in glioblastoma (575 and 195 PSs, respectively) (Table 2). Because 55–60% of the overexpressed genes in both cancers were tissue-selective genes, the number of overexpressed tissue-selective genes in astrocytoma was 2.6-fold higher than in glioblastoma (319 and 122 PSs, respectively) (SI Table 8). Most of the tissue-selective genes overexpressed in glioblastoma (104 of 122 PSs) are also overexpressed in astrocytoma (SI Table 8). The number of overexpressed tissue-selective genes unique to astrocytoma is thus ≈12-fold higher than those that are unique to glioblastoma (215 and 18 PSs, respectively). These results indicate that although both brain cancer types are of astrocytic origin, astrocytoma overexpress a much higher number of tissue-selective genes than glioblastoma.

Cancers Overexpress Only a Few Genes That Are Selectively Expressed in the Same Tissue in Which the Cancer Originated. We showed (7) that the tissue-selective genes that are overexpressed in human leukemias were rarely preferentially expressed in hematopoietic tissues. We have now determined in human solid cancers the frequency of overexpressed genes that show tissue selectivity for the same tissue from which the cancer originated. The results show that only 6 of the 195 overexpressed PSs in glioblastoma (≈3%) show selective expression in normal brain (Table 2). In all other cancer types, including the brain tumor astrocytoma, the frequency of overexpressed PSs showing a tissue-selective expression in the same tissue from which the cancer originated was even lower (0–1%) (Table 2). When all cancers are considered, <0.3% of their overexpressed genes show a tissue-selective expression in the same tissue in which the cancer originated. Our results indicate that the solid cancers overexpress genes that are selectively expressed in tissues other than the cancers' tissue of origin and do not tend to overexpress genes that are normally selectively expressed in the same tissue in which the cancer originated.

Discussion

Different studies on gene expression, by using DNA microarrays containing tens of thousands of genes, have shown that only several hundred genes are either overexpressed or underexpressed in various cancers compared with their normal tissue of origin (11–17). Thus the level of expression of most genes is not significantly altered in cancer cells compared with their tissue of origin, which allows the identification of the tissue origin of even distant metastases (18). In some cancers, such as melanoma, the cancer cells are derived from melanocytes, a minor cell population in normal skin. Our results indicate that even in this case, the expression level of ≈97% of the genes in melanoma did not exceed that of normal skin. In addition, analysis of gene expression in melanoma and benign nevi, which have a similar melanocyte content, identified 33 genes that were overexpressed >10-fold in melanoma, and these genes were also overexpressed to a similar extent in melanoma compared with normal skin (19). Therefore, by comparing gene expression level in the cancer and its normal tissue of origin, by using our stringent criteria for overexpression, it can be assumed that most of the overexpressed genes are expressed by the cancer cells.

Genome-wide analysis of transcription profiles in various normal human tissues revealed tissue-selective differences in gene-expression levels (20, 21). We showed (6, 7) that mouse and human leukemia cells overexpressed genes whose normal expression profile was selective for various nonhematopoietic tissues. Based on publicly available gene-expression data sets, including 566 samples from 13 different normal human tissues and 1,401 samples from 12 different types of solid cancers that originated in these tissues, we have now determined the expression profile in different normal tissues of those genes that are overexpressed in these cancers. We found that 1,340 of the 2,929 PSs that are overexpressed at least 4-fold in cancers (≈45%) have a tissue-selective expression profile in various normal human tissues, whereas the remaining ≈55% of the cancer overexpressed genes did not show such a tissue-selective expression in the tissues we analyzed. Our analysis did not include normal tissues such as muscle, pancreas, stomach, and bladder, and some of the cancer overexpressed genes we have listed as not tissue-selective, may be tissue-selective for other tissues. Less than 0.3% of the overexpressed genes in the different solid cancers were selectively expressed in the normal tissue in which the cancer originated. Thus, as in leukemias (7), solid cancers only rarely overexpress genes whose expression profile is selective for the normal tissue in which the cancer originated.

Solid cancers contain a variety of normal cell types including cells of the immune system. Had there been a major contribution of normal immune cells to the genes overexpressed in

solid cancers, it could be expected that a large proportion of overexpressed genes in many solid cancers would belong to the blood-selective group of genes. However, our analysis indicates (Table 2) that in lung cancer, for example, only 9 of the 465 overexpressed genes are blood-selective, whereas the number of other tissue-selective genes is much higher. In none of the cancers examined are blood-selective genes the most prevalent tissue-selective group among the overexpressed genes. In addition, we showed (7) that human SW480 colon carcinoma cell line overexpresses many blood-selective genes. The results indicate that most of the overexpressed genes are expressed by the cancer cells. The results with leukemias (1, 7) and the present results with solid cancers indicate that the ability to overexpress genes that are selectively expressed in tissues other than the cancer's tissue of origin, is a general property of cancer cells that may play an important role in determining the cancers' behavior by promoting cancer cell viability, growth, and metastasis (1).

We found that different cancer types had differences in the number of overexpressed genes that are selectively expressed in normal tissues that are different from the tissue in which the cancer originated. Such differences were found even between two different brain cancers, astrocyoma and glioblastoma, where the number of overexpressed tissue-selective PSs unique to astrocytomas was ≈12-fold higher than those unique to glioblastomas. Analysis of the 1,340 tissue-selective and cancer-overexpressed PSs indicated that testis-selective genes showed the highest (61%) and brain-selective genes showed the lowest (14%) frequency of genes overexpressed in two or more cancer types. Of all overexpressed genes in cancers, colon cancer and melanoma showed the highest (81%) and lowest (17%) frequencies of tissue-selective genes, respectively, and in all other cancer types this frequency ranged from 40% to 65%. Melanomas overexpressed the highest number of brain-selective genes (45 PSs) compared with all other cancer types. It is suggested that overexpression of brain-selective genes in melanoma may contribute to the involvement of brain in melanoma metastases (22). The neural-crest origin of melanocytes may also contribute to the expression of brain-selective genes in melanoma. Although colon cancers showed the highest frequency of overexpressed genes that are tissue-selective for various noncolon tissues, and testis-selective genes showed the highest frequency of genes overexpressed in two or more cancer types, colon cancer did not overexpress any testis-selective gene (Table 2). The lack of overexpression of testis-selective genes was also found in prostate cancers (Table 2). It will be interesting to determine how this lack of overexpression of testis-selective genes contributes to the behavior of colon and prostate cancers.

The results described above were obtained by using primary cancers, raising the question of whether cancer stem cells isolated from such cancers give similar results. A recent DNA microarray study with human $CD44^+CD24^-$/low cells isolated from human breast cancer, which can initiate cancer in immunodeficient mice, identified 186 genes overexpressed at least 2-fold compared with normal breast epithelium (23). In the breast cancer data sets we have analyzed, 90 genes were overexpressed at least 4-fold compared with normal breast epithelium (Table 2) and 162 genes were overexpressed at least 2-fold. Of the 186 genes overexpressed 2-fold in the isolated $CD44^+CD24^-$/low cells, we detected 40 genes as tissue-selective for various normal tissues. In primary breast cancers, none of the 4-fold overexpressed genes was selectively expressed in normal breast (Table 2), and in the isolated $CD44^+CD24^-$/low cells only 3 of the 186 genes (1.6%) are selectively expressed in normal breast tissue. Assuming that the isolated $CD44^+CD24^-$/low cells represent breast cancer stem cells, these results indicate that regarding the number of overexpressed genes, the frequent overexpression of nonbreast tissue-selective genes and the rare overexpression of breast-selective genes, primary breast cancer and these isolated breast cancer stem cells appear to be similar. It will be interesting to determine whether cancer stem cells isolated from other cancers also behave in the same way.

Materials and Methods

Data Sets. The database was compiled of 20 data sets of Affymetrix (Santa Clara, CA) U133A or U133 Plus 2 DNA microarray data of 13 normal tissues and 12 cancer types described in Tables 1 and 2 (18–20, 24–35). All data sets were downloaded from Gene Expression Omnibus (36) in MICROARRAY SUITE software, Version 5 preprocessed format, by using the accession numbers described in SI Table 4. The database contains a total of 1,967 samples of which 1,401 samples are late-stage solid cancers, and 566 samples are normal tissue samples. The details of how many samples for each normal tissue and the different types of cancer are shown in SI Table 4. The 21,700 PSs which where found to be present in all samples of normal tissues and cancers were selected for analysis. Noise levels were assessed by comparing samples within groups. If samples within groups deviated from an average 2-fold noise spread around the diagonal, these samples were discarded. In this way, overall noise levels are kept at a level comparable to other similar large-scale studies (18, 20).

Processing. PS expression values <10 were adjusted to 10 to eliminate noise from the data. The often-observed nonlinear variation in background intensity when performing pairwise comparisons of microarray data can easily be corrected by a lowess correction in the ratio-intensity transformed representation (37, 38). For supervised testing of several groups against each other, the requirement becomes that all samples share the same nonlinearity in background intensity. To correct for variances in the background intensity mapping between different data sets, we used a slight modification of the mean cyclic lowess correction (39, 40). Our modification is that we correct the background variability of all samples to an arbitrarily chosen single preselected sample instead of to the total database mean (*SI Appendix*). The only implication of this choice is that computation time is longer. However, the tradeoff is that adding extra data samples is fast, because only the recently added samples need the correction versus the whole database as required for the mean cyclic lowess method. This correction introduces only minor negligible artifacts. For example, for small corrections the method almost exactly preserves sample ordering upon SPIN-sorting (41). This result shows that no significant artifacts are introduced from either systematic global or local noise mixing in the correction procedure.

Selection of Genes Showing Significant Differences in Expression Between Any Two Groups of Samples. The supervised tests were two-tailed Student's t tests with 5% false-discovery rate-management (42), followed by a constraint of a 4-fold difference of the means. In the final step, only genes with a mean value 60% above the sample median in the test group were retained. This rather conservative filter yields reliable and biologically meaningful sets of discovered PSs while keeping the numbers manageable.

This work was supported by the Benoziyo Institute of Molecular Medicine and the Dolfi and Lola Ebner Center for Biomedical Research (L.S. and J.L.); the Fraenkel Foundation and the Statistical Physics of Information Processing and Combinatorial Optimization (STIPCO) Network of the Research Training Network programme of the European Commission Contract HPRN-CT-2002–00319 (to J.B.A.); and the Ridgefield Foundation and the Israel Ministry of Science (E.D.).

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