Systematic analysis of the achaete-scute complex-like gene signature in clinical cancer patients

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Abstract. The achaete-scute complex-like (ASCL) family, also referred to as 'achaete-scute complex homolog' or 'achaete-scute family basic helix-loop-helix transcription factor', is critical for proper development of the nervous system and deregulation of ASCL plays a key role in psychiatric and neurological disorders. The ASCL family consists of five members, namely ASCL1, ASCL2, ASCL3, ASCL4 and ASCL5. The ASCL1 gene serves as a potential oncogene during lung cancer development. There is a correlation between increased ASCL2 expression and colon cancer development. Inhibition of ASCL2 reduced cellular proliferation and tumor growth in xenograft tumor experiments. Although previous studies demonstrated involvement of ASCL1 and ASCL2 in tumor development, little is known on the remaining ASCL family members and their potential effect on tumorigenesis. Therefore, a holistic approach to investigating the expression of ASCL family genes in diverse types of cancer may provide new insights in cancer research. In this study, we utilized a web-based microarray database (Oncomine; www.oncomine.org) to analyze the transcriptional expression of the ASCL family in clinical cancer and normal tissues. Our bioinformatics analysis revealed the potential involvement of multiple ASCL family members during tumor onset and progression in multiple types of cancer. Compared to normal tissue, ASCL1 exhibited a higher expression in cancers of the lung, pancreas, kidney, esophagus and head and neck, whereas ASCL2 exhibited a high expression in cancers of the breast, colon, stomach, lung, head and neck, ovary and testis. ASCL3, however, exhibited a high expression only in breast cancer. Interestingly, ASCL1 expression was downregulated in melanoma and in cancers of the bladder, breast, stomach and colon. ASCL2 exhibited low expression levels in sarcoma, melanoma, brain and prostate cancers. Reduction in the expression of ASCL3 was detected in lymphoma, bladder, cervical, kidney and epithelial cancers. Similarly, ASCL5 exhibited low expression in the majority of brain cancer subtypes, such as glioblastoma and oligodendroglioma. This analysis supports the hypothesis that specific ASCL members may play an important role in cancer development. Collectively, our data suggest that alterations in the expression of ASCL gene family members are correlated with cancer development. Furthermore, ASCL family members were categorized according to cancer subtype. The aim of this report was to provide novel insights to the significance of the ASCL family in various cancers and our findings suggested that the ASCL gene family may be an ideal target for future cancer studies.

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Introduction

Cancer is the leading cause of morbidity and mortality worldwide according to the data of the International Agency for Research on Cancer (https://www.iarc.fr/), updated February, 2015. In 2012, there were an estimated 14 million new cancer cases and 8.2 million cancer-related deaths (1). Multiple studies indicate that the most prevalent cancers are lung (1.59 million deaths), liver (745,000 deaths), stomach (723,000 deaths), colorectal (694,000 deaths), breast (521,000 deaths) and esophageal cancer (400,000 deaths) (1). Since aberrations in the transcriptional expression are known to cause cancer, a

primary approach to understanding cancer is to identify oncogenic genes and elucidate their roles in cancer regulation (2).

The achaete-scute complex-like (ASCL) gene family, also referred to as 'achaete-scute complex homolog' or 'achaete-scute family basic helix-loop-helix transcription factor' and mammalian achaete-scute homologues (MASH), comprises five family members (ASCL1-ASCL5; Table I) (3,4). All ASCL genes encode basic helix-loop-helix transcription factors that control the development of the nervous system (2,3). Given the involvement of ASCL in neuroblast cell fate determination, the ASCL family members are also referred to as proneural genes. The function of the ASCL gene family is highly conserved across all vertebrates; however, ASCL family gene expression and their effect on target cells are not restricted to the nervous system. For example, expression of ASCL family members is detected in progenitor cells during muscle and gut cell differentiation (5-7). These findings emphasize the significance of ASCL genes during organogenesis. However, whether ASCL family members play an integral role in cancer initiation and progression has not been fully elucidated.

ASCL1 is briefly expressed during nervous system development, including olfactory and autonomic neural development (3). ASCL1 is also detected in sympathetic neurons during early embryonic stages in humans (4). In addition to its role during development, ASCL1 overexpression has been associated with human neuroendocrine cancers. However, whether ASCL1 plays a role in the initiation and progression of other cancers remains unclear (8,9).

ASCL2 (HASH2) is expressed by trophoblasts during placental development (10). Recent data suggest that ASCL2 may affect the Wnt signaling pathway. The ASCL2 may form a complex with the Wnt pathway signal transducer β-catenin in order to synergistically activate the expression of downstream target genes (11,12). Moreover, ASCL2 may modulate the plasticity between epithelial and mesenchymal characteristics in colon cancer (13).

Little is known on the function of the remaining ASCL family members. ASCL3 is expressed in adult progenitor cells that mature into acinar- and duct-type cells in murine salivary glands (14). ASCL4 may play a role during skin development and it exhibits a 7-fold higher expression levels in fetal skin compared with adult skin (15). At present, the mechanism and function of ASCL5 are yet to be determined. Although previous studies describe a developmentally significant role for the ASCL gene family, our overall understanding of their function during development and their potential roles during tumorigenesis is incomplete.

Major strives have been made to catalog the mRNA expression profiles of numerous cancers in vast databases. One advantage of these massive resources is to increase our ability to identify potential biomarkers in specific tumors and to characterize their molecular signatures. Since tumor initiation coincides with alterations in normal gene expression, analysis of the differential gene expression in tumor cells may reveal unique tumor biomarkers. Thus, these databases, particularly the Oncomine microarray database (16), were utilized to gain a better understanding of the ASCL family role in the initiation and progression of several tumors, aiming to provide useful insights in prospective research into cancer association with the ASCL gene family.

Materials and methods

Meta-analysis. A meta-analysis was used to analyze the mRNA expression of the ASCL family in clinical cancer specimens by following the PRISMA guidelines (17,18) Oncomine (www.oncomine.org), a web-based microarray database, was used to analyze the mRNA expression of ASCL in clinical cancer tissue (19). According to 'Oncomine Platform Overview Q1 2014,' the database resource of Oncomine includes upwards of 700 independent datasets with an estimated 90,000 microarray trials. Oncomine has standardized and organized the datasets of public cancer microarray data into different cancer type and subtypes (16,20).

ASCL gene expression. ASCL gene (ASCL1-5) expression in 20 cancer types was investigated. Detailed information on ASCL genes, such as tissue of origin, comparing mRNA expression with matched normal tissue types, was displayed in groups. The gene summary view in Oncomine was presented throughout the analysis with an alteration in color, reflecting the degree of expression. The expression coloration represents a gene with highest ranking in a particular type of cancer based on the threshold analysis (Fig. 1).

Statistical analysis. The cancer vs. normal filter that only displayed datasets investigating ASCL gene mRNA expression in the same tissue of origin was selected. To be included in the study, all the data had to satisfy the threshold with a P-value of <0.01, a fold change of >1.5 and a gene rank percentile of <10%. Statistical analyses were performed using the Oncomine default algorithms, such as P-values, two-tailed Student's *t*-test, and multiple testing corrections.

Results and Discussion

Analysis of ASCL expression in various tumors. Several studies have identified potential roles for ASCL family members in cancer development; however, our overall understanding of ASCL family member function during tumor initiation and progression is incomplete. To investigate a potential alteration in ASCL family gene expression in different types of cancer, we accessed the web-based Oncomine microarray database to analyze 20 different types of cancer. Cancer tissue was compared with normal tissue (control) and thresholds were set to screen suitable datasets from the Oncomine database. To include suitable datasets for further analysis, the gene expression in cancer cells compared with that of normal tissue had to fulfill the following threshold criteria: Fold change >1.5, P<0.01 and gene rank percentile <10% (Fig. 1). Our analysis demonstrated alterations in ASCL gene family expression in multiple cancer types, which may provide useful information for future studies investigating the role of ASCL genes in tumorigenesis.

ASCL1. The proneural transcriptional factor ASCL1/MASH1 is essential for proper nervous system development (21). In the cerebrum, ASCL1 controls the primitive as well as the late phases of neurogenesis, with the division of radial glia progenitors and the radial migration of post-mitotic neurons (22,23). ASCL1 controls the expression of numerous target genes that are involved in cell cycle progression and cytoskeletal

Table I. Function of the achaete-scute complex-like family members.

Official symbol	Alias	Biological function	(Refs.)
ASCL1	ASH1,	Regulation of transcription from RNA polymerase II promoter	(37)
	HASH1,	Cerebral cortex GABAergic interneuron differentiation	(38)
	MASH1,	Sympathetic nervous system development	(39)
	bHLHa46	Negative regulation of apoptotic process	(40)
		Noradrenergic neuron fate commitment	(41)
		Lung epithelial cell differentiation	(42)
		Notch signaling pathway	(43)
		Response to retinoic acid	(37)
		Neurogenesis	(44)
ASCL2	ASH2,	Regulation of transcription from RNA polymerase II promoter	(45,46)
		Spongiotrophoblast differentiation	(47)
	HASH2,	*In utero* embryonic development	(10)
		Sequence-specific DNA binding	(45)
	MASH2,	Somatic stem cell maintenance	(9)
		Placenta development	(48)
	bHLHa45		
ASCL3	SGN1,	RNA polymerase II regulatory region sequence-specific DNA binding	(49)
		Regulation of transcription from RNA polymerase II promoter	(49)
	HASH3, bHLHa42	Transcription factor complex	(49)
ASCL4	HASH4,	Regulation of transcription from RNA polymerase II promoter	(15)
		Skin development	(15)
	bHLHa44	Protein binding	(50)
ASCL5	bHLHa47	Regulation of transcription, DNA-template	(33)

ASCL, achaete-scute complex-like; bHLH, basic helix-loop-helix.

reorganization associated with neuronal cell migration (6,7). Recently, a potential oncogenic role for ASCL1 in lung cancer has been reported (23); however, the role of ASCL1 in cancer remains unclear. Our analysis indicated that ASCL1 is significantly overexpressed in the majority of cancer types, such as cancers of the brain, lung, head and neck, prostate, pancreas, kidney, esophagus, leukemia, lymphoma and sarcoma (Fig. 1). ASCL1 also ranked in the top 1% of overexpressed genes in leukemia, brain and lung cancer. Importantly, our analysis indicated that ASCL1 is overexpressed in the majority of brain cancers, such as glioblastoma, oligodendroglioma, anaplastic astrocytoma, anaplastic oligodendroglioma and anaplastic oligoastrocytoma. Compared with normal tissue, ASCL1 exhibited a higher expression in brain tumor tissues (P-values of 0.004-2.50E-21), and the ASCL1 gene ranked 1-7% in our meta-analysis results (Table II). We found that, in various lung cancers, such as small-cell lung carcinoma and carcinoid tumors, ASCL1 was significantly overexpressed (P-values of 0.002-3.53E-13) and the gene ranked in the top 1-8% relative to the control. In addition to brain tumors, ASCL1 was also highly expressed in acute adult T-cell leukemia/lymphoma, with the gene ranking in the top 1%, a 3.76-fold change, and a P=3.43E-5. These data indicated that ASCL1 expression varied in different types of leukemia. We also observed that ASCL1 was highly expressed in leiomyosarcoma (3.55-fold change, P=6.13E-4 and gene ranking in the top 7%), prostate carcinoma (3.21-fold change, P=0.001 and gene ranking in the top 1%), pancreatic adenocarcinoma (3.22-fold change, P=0.002 and gene ranking in the top 6%), renal oncocytoma (5.16-fold change, P=0.002 and gene ranking in the top 8%) and Barrett's esophagus (1.95-fold change, P=0.002 and gene ranking in the top 9%) (Table II).

In contrast to brain cancer and lymphomas, other cancers exhibited a reduction in ASCL1 expression. Gastric cancer and melanoma were among the top 1% of tumors that exhibited ASCL downregulation. The reduction in the ASCL1 transcript level suggested a tumor suppressor role, since tumor suppressor genes tend to exhibit a low or reduced expression in tumor tissue compared with normal tissue. Our analysis indicated lower ASCL1 expression in gastric, bladder and lung cancers. Evidence of this trend is also supported by a previous study that specifically evaluated a tumor suppressor gene in breast cancer datasets from the Oncomine database, which revealed a significant downregulation and low expression of the tumor suppressor gene ADAMTS1 in breast carcinomas when compared with normal tissue (24). Another similar study on the SIRT3 tumor suppressor gene also revealed lower expression in various tumor types (25). Given the pattern of downregulation, we hypothesized that ASCL1 may also play a tumor suppressor role in a subset of tissues. ASCL1 expression was considerably downregulated in lymphoma (diffuse large B-cell lymphoma, primary effusion lymphoma and mantle cell

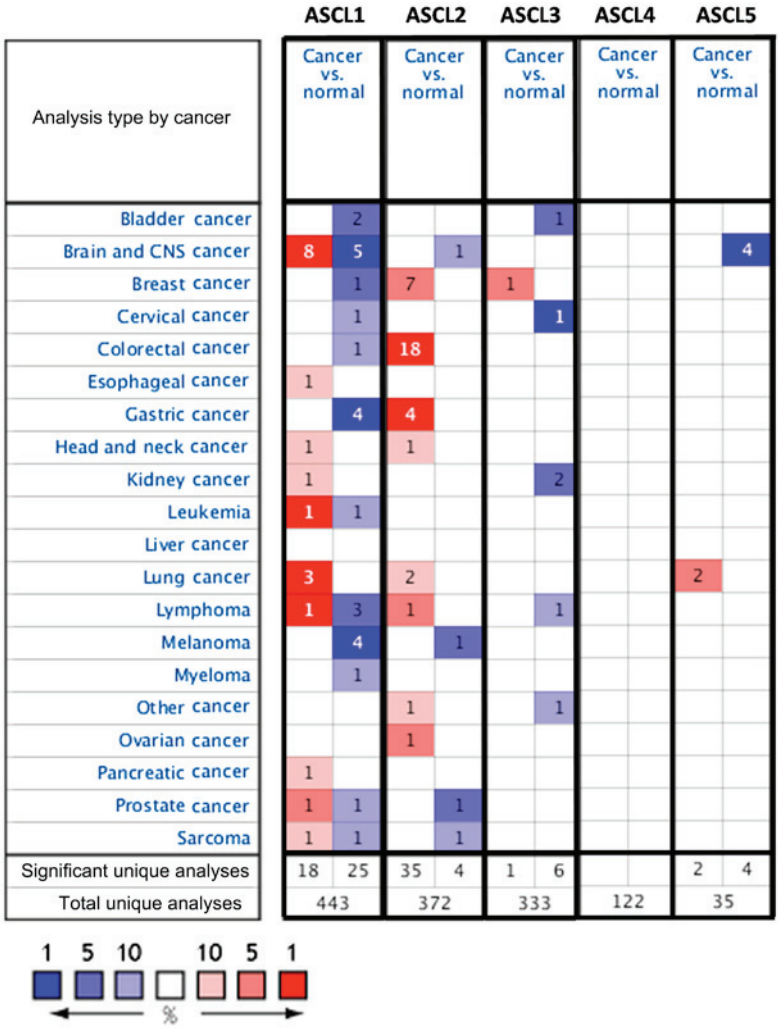

Figure 1. Expression of ASCL family genes in 21 types of cancer compared with normal tissue controls. The gene name of each channel is shown. Each gene was found in the tissue of origin, and the color gradient correlates with decreasing gene rank percentile. The search criteria threshold was set at P<0.01 with a fold change of >1.5 and a gene rank percentile of <10% for screening microarray datasets of cancer vs. normal cases. Cell color is determined by the best gene rank percentile for the analyses within the cell. Note: An analysis may be counted in more than one cancer type. ASCL, achaete-scute complex-like; CNS, central nervous system.

lymphoma). The reduction in ASCL1 expression ranged from -1.61- to -2.08-fold change, with P-values of 9.25E-4-1.15E-6 and the gene ranking in the top 3-10%. Our bioinformatics analyses of gastric cancer revealed that ASCL1 exhibited a lower expression in the majority of gastric cancer subtypes, namely gastric mixed adenocarcinoma, gastrointestinal stromal tumors and gastric intestinal-type adenocarcinoma. The ASCL1 expression ranged from -1.60- to -3.64-fold downregulation, with P-values of 0.002-1.06E-6 and the gene ranking in the top 1-8%. ASCL1 exhibited a lower expression in most types of melanoma, namely cutaneous melanoma, non-neoplastic nevus, benign melanocytic skin nevus and monoclonal gammopathy of undetermined significance. The ASCL1 transcript expression ranged from -1.64- to -3.22-fold downregulation, with P-values of 0.003-2.27E-5 and the gene ranking in the top 1-10%. ASCL1 also exhibited a lower expression in bladder cancer (-1.77-fold change, P=2.36E-6 and gene ranking in the top 6%), invasive ductal breast carcinoma (-1.59-fold change, P=5.03E-6 and gene ranking in the top 3%) and colon cancer (-3.58-fold change, P=9.57E-6 and gene ranking in the top 7%) (Table II). These analyses suggest that the effect of ASCL1 downregulation on transcript expression may be an equally important alteration as increased expression in cancer biology. Interestingly, ASCL1 was found to be both up- and downregulated in brain tumors compared with normal tissue. The conflicting expression profiles of ASCL1 in the same type of cancer may be due to the wide-ranging categories for each of the cancer subtypes (Table II). This discrepancy may be a sample size issue arising from the original publications' reported data including a low number of samples from these tumor types. Collectively, our data suggest that alterations in ASCL1 expression may adversely affect tissue homeostasis, which may result in tumorigenesis.

ASCL2. ASCL2 is a basic helix-loop-helix transcription factor that is expressed in neuronal precursors (26). ASCL2 is a target of the Wnt signaling pathway and previous studies indicated that ASCL2 may regulate LGR5 in intestinal stem cells in response to Wnt signaling (9,27). Moreover, ASCL2 is involved in T-helper cell (TH) 1 and TH17 differentiation (28).

ASCL2 is strongly expressed in colon cancer tissues and cell lines (HT-29 and LS174T cells). Selective blockade of

Table II. Achaete-scute complex-like (ASCL) 1 expression in cancer.

Gene	Cancer	Subtype	Case no.	Change expression	P-value (cancer/ normal)	*t*-test (cancer/ normal)	Fold change (cancer/ normal)	% Gene ranking	Database reference
ASCL1	Brain	Oligodendroglioma	180	↑	2.50E-21	13.45	4.40	5 (top 1%)	(51)
		Oligodendroglioma	54	↓	4.68E-4	-5.78	-1.81	316 (top 3%)	(52)
		Glioblastoma	180	↑	4.68E-11	7.83	3.64	1,181 (top 7%)	(51)
		Glioblastoma	38	↓	3.87E-8	-7.30	-1.63	61 (top 1%)	(53)
		Glioblastoma	101	↓	6.47E-8	-7.81	-5.48	1,186 (top 7%)	(54)
		Anaplastic astrocytoma	180	↑	1.45E-10	9.58	5.30	86 (top 1%)	(51)
		Glioblastoma	84	↑	1.06E-11	10.48	3.38	244 (top 2%)	(55)
		Anaplastic oligodendroglioma	33	↑	1.01E-9	8.89	5.22	71 (top 1%)	(56)
		Brain glioblastoma	557	↑	1.06E-9	12.86	4.32	546 (top 5%)	(57)
		Anaplastic oligoastrocytoma	54	↑	0.001	4.52	2.99	283 (top 2%)	(52)
		Anaplastic oligodendroglioma	54	↑	0.004	4.37	1.57	501 (top 4%)	(52)
		Oligoastrocytoma	38	↓	3.60E-4	-12.58	-1.90	30 (top 1%)	(53)
		Astrocytoma	42	↓	3.96E-4	-5.16	-1.51	184 (top 3%)	(58)
	Lung	Small-cell lung carcinoma	203	↑	3.53E-13	17.89	576.45	2 (top 1%)	(59)
		Lung carcinoid tumor	203	↑	0.002	3.15	5.56	641 (top 8%)	(59)
		Small-cell lung carcinoma	73	↑	1.43E-5	8.49	18.57	20 (top 1%)	(60)
	Leukemia	Acute adult T-cell leukemia/lymphoma	47	↑	3.43E-5	4.75	3.76	99 (top 1%)	(61)
		Chronic lymphocytic leukemia	111	↓	5.31E-4	-4.27	-3.07	814 (top 10%)	(62)
	Lymphoma	Diffuse large B-cell lymphoma	336	↓	1.15E-6	-5.28	-1.61	180 (top 3%)	(63)
		Primary effusion lymphoma	336	↓	9.25E-4	-4.17	-2.08	792 (top 10%)	(63)
		Mantle cell lymphoma	336	↓	9.25E-4	-4.18	-1.83	223 (top 3%)	(63)
	Head and neck	Salivary gland adenoid cystic carcinoma	22	↑	4.89E-4	6.19	62.22	674 (top 8%)	(64)
	Sarcoma	Leiomyosarcoma	54	↑	6.13E-4	3.80	3.55	777 (top 7%)	(65)
		Gastrointestinal stromal tumor	90	↓	9.50E-5	-4.67	-1.57	1,356 (top 8%)	(66)
	Prostate	Prostate carcinoma	35	↑	0.001	4.99	3.21	179 (top 3%)	(67)
		Prostate carcinoma	122	↓	4.19E-5	-4.25	-1.60	1,743 (top 10%)	(68)
	Pancreas	Pancreatic adenocarcinoma	36	↑	0.002	4.43	3.22	760 (top 6%)	(69)
	Kidney	Renal oncocytoma	67	↑	0.002	4.17	5.16	1,540 (top 8%)	(70)
	Esophagus	Barrett's esophagus	48	↑	0.002	3.09	1.95	1,283 (top 9%)	(71)
	Gastric	Gastric mixed adenocarcinoma	90	↓	1.06E-6	-6.44	-1.63	133 (top 1%)	(66)
		Gastric intestinal-type adenocarcinoma	69	↓	1.29E-6	-5.29	-3.64	357 (top 2%)	(72)
		Gastric cancer	27	↓	0.002	-3.18	-2.68	583 (top 3%)	(73)
		Gastric cancer	160	↓	0.004	-2.71	-1.60	1,365 (top 8%)	(74)

Table II. Continued.

Gene	Cancer	Subtype	Case no.	Change expression	P-value (cancer/ normal)	*t*-test (cancer/ normal)	Fold change (cancer/ normal)	% Gene ranking	Database reference
	Bladder	Superficial bladder cancer	60	↓	2.36E-6	-6.16	-1.77	721 (top 6%)	(75)
		Infiltrating bladder urothelial carcinoma	60	↓	4.20E-6	-5.60	-1.83	555 (top 5%)	(75)
	Breast	Invasive ductal breast carcinoma	64	↓	5.03E-6	-9.33	-1.59	300 (top 3%)	(76)
	Colon	Colon adenoma	64	↓	9.57E-6	-4.72	-3.58	1,274 (7%)	(77)
	Melanoma	Melanoma	37	↓	2.27E-5	-17.47	-3.22	37 (top 1%)	(78)
		Non-neoplastic nevus	37	↓	0.003	-3.57	-1.67	435 (top 6%)	(78)
		Benign melanocytic skin nevus	70	↓	4.15E-5	-4.98	-2.71	382 (top 4%)	(79)
		Cutaneous melanoma	87	↓	1.32E-4	-4.76	-2.77	470 (top 3%)	(80)
	Myeloma	Monoclonal gammopathy of undetermined significance	78	↓	1.42E-4	-3.84	-1.64	1,293 (top 7%)	(62)

ASCL2 disrupts tumor cell proliferation and migration in tumor xenograft models (10,29,30), a result consistent with our bioinformatics analysis (Fig. 1). This is particularly true in colon cancer tissues compared with normal tissues; however, whether ASCL2 plays a role in initiation and progression of other tumor types remains unclear.

ASCL2 expression was altered in 8 of 21 investigated cancers and was commonly observed in colorectal, gastric, breast, ovarian, testicular, lung and head and neck cancers, as well as lymphoma (Fig. 1). However, based on our bioinformatics analysis, our results were strikingly different. Downregulation of ASCL2 was only observed in the top 5% and 9% of underexpressed genes in melanoma, and brain and gastric cancers, respectively (Fig. 1).

Our analysis revealed that ASCL2 expression is significantly upregulated in various breast cancer subtypes, such as invasive ductal, invasive lobular and medullary breast carcinoma, with P-values of 0.009-4.39E-72, gene ranking 2-10% and a fold change of 1.66-14.9 compared with normal tissues (Table III). In colorectal tumors, such as adenocarcinoma of the colon, rectum, cecum or rectosigmoid region, colonic adenoma, rectal adenoma, colon adenoma epithelia and colon carcinoma epithelia, ASCL2 also exhibited significant upregulation compared with normal tissues, with P-values of 3.60E-7-8.24E-52, gene ranking 1-9% and a fold change of 5.64-31.35 (Table III).

In gastric cancers, such as diffuse gastric adenocarcinoma, gastric intestinal-type adenocarcinoma and gastric mixed adenocarcinoma, ASCL2 exhibited significant upregulation compared with normal tissues, with P-values of 6.30E-4-1.74E-6, gene ranking 1-5% and a fold change of 2.35-4.45. Additionally, we found that ASCL2 is highly expressed in squamous cell lung carcinoma (1.84-fold change, P=7.81E-8 and gene ranking in the top 9%), nodular lymphocyte-predominant Hodgkin's lymphoma (2.65-fold change, P=7.61E-5 and gene ranking in the top 5%), nasopharyngeal carcinoma (1.56-fold change, P=5.08E-4 and gene ranking in the top 10%), ovarian endometrioid adenocarcinoma (1.76-fold change, P=0.001 and gene ranking in the top 3%); testicular seminoma (3.21-fold change, P=0.007 and gene ranking in the top 6%) (Table III).

Of note, lower ASCL2 gene expression levels were found in certain cancer subtypes, such as brain and gastric cancer, and melanoma. These subtypes included oligodendroglioma (-1.73-fold change, P=4.42E-4 and gene ranking in the top 9%), gastrointestinal stromal tumors (-2.59-fold change, P=4.59E-4 and gene ranking in the top 9%), and cutaneous melanoma (-6.74-fold change, P=6.22E-4 and gene ranking in the top 5%) (Table III). Thus, ASCL2 exhibited increased mRNA expression in some cancer tissues and decreased expression in others. Overall, our analysis indicated that ASCL2 was ranked in the top 10% of genes involved in the regulation of breast, colorectal, lung, gastric, head-neck, ovarian and testicular cancers and lymphoma, whereas in brain cancer and melanoma it exhibited significant downregulation compared with normal tissue (Table III). These findings indicate that cell context-specific alterations in ASCL2 expression may play a critical role in cancer biology.

ASCL3. ASCL3 (Sgn1) belongs to the MASH gene family of transcription factors that has been associated with cell fate

Table III. Achaete-scute complex-like (ASCL) 2 expression in cancer.

Gene	Cancer	Subtype	Case no.	Change expression	P-value (cancer/ normal)	*t*-test (cancer/ normal)	Fold change (cancer/ normal)	% Gene ranking	Database reference
ASCL2	Breast	Invasive ductal breast carcinoma	2136	↑	4.39E-72	23.54	2.14	582 (top 4%)	(81)
		Invasive lobular breast carcinoma	2136	↑	1.42E-26	12.34	2.31	1,080 (top 6%)	(81)
		Invasive ductal and invasive lobular breast carcinoma	2136	↑	4.26E-16	9.38	2.05	1,794 (top 10%)	(81)
		Medullary breast carcinoma	2136	↑	5.67E-12	9.87	2.84	449 (top 3%)	(81)
		Breast carcinoma	2136	↑	1.88E-4	4.60	1.67	1,375 (top 8%)	(81)
		Invasive breast carcinoma stroma	59	↑	1.49E-14	11.34	3.91	1,068 (top 6%)	(82)
		Ductal breast carcinoma *in situ*	63	↑	0.009	4.18	14.91	289 (top 2%)	(83)
	Colorectal	Rectal adenocarcinoma	130	↑	8.24E-52	26.18	9.71	10 (top 1%)	(84)
		Colon adenocarcinoma	237	↑	3.20E-27	15.78	6.53	125 (top 1%)	(98)
		Rectal adenocarcinoma	237	↑	3.36E-27	16.38	8.77	66 (top 1%)	(98)
		Cecum adenocarcinoma	237	↑	1.97E-12	10.84	6.28	396 (top 2%)	(98)
		Rectosigmoid adenocarcinoma	237	↑	2.31E-8	18.07	6.50	206 (top 2%)	(98)
		Colon adenocarcinoma	105	↑	1.36E-26	23.35	8.96	2 (top 1%)	(85)
		Cecum adenocarcinoma	105	↑	8.03E-8	8.60	7.15	274 (top 2%)	(85)
		Rectal adenocarcinoma	105	↑	1.02E-6	12.69	6.64	50 (top 1%)	(85)
		Rectosigmoid adenocarcinoma	105	↑	1.87E-4	5.47	6.95	1,632 (top 9%)	(85)
		Colorectal carcinoma	82	↑	3.55E-19	15.43	24.81	46 (top 1%)	(86)
		Colorectal adenocarcinoma	105	↑	2.63E-18	12.56	6.13	62 (top %)	(87)
		Colorectal carcinoma	105	↑	1.72E-13	9.40	5.64	100 (top 1%)	(87)
		Colon adenoma	64	↑	5.60E-18	15.27	8.94	101 (top 1%)	(77)
		Rectal adenoma	64	↑	3.80E-5	7.84	17.43	1,580 (top 9%)	(77)
		Colon adenoma	40	↑	1.80E-11	21.19	31.35	17 (top 1%)	(87)
		Colon carcinoma	40	↑	1.05E-10	20.53	24.23	162 (top 1%)	(87)
		Colon adenoma epithelia	40	↑	5.68E-8	10.74	14.14	233 (top 2%)	(87)
		Colon carcinoma epithelia	40	↑	3.60E-7	10.42	10.79	914 (top 5%)	(87)
	Lung	Squamous cell lung carcinoma	156	↑	7.81E-8	6.48	1.84	1,660 (top 9%)	(88)
		Squamous cell lung carcinoma	203	↑	0.004	2.79	3.63	639 (top 8%)	(59)
	Gastric	Diffuse gastric adenocarcinoma	90	↑	1.74E-6	5.35	3.40	348 (top 2%)	(66)
		Gastric intestinal-type adenocarcinoma	90	↑	3.01E-6	5.68	4.17	131 (top 1%)	(66)
		Gastric mixed adenocarcinoma	90	↑	6.30E-4	4.35	4.45	923 (top 5%)	(66)
		Gastric cancer	160	↑	9.27E-6	4.43	2.35	400 (top 3%)	(74)
		Gastrointestinal stromal tumor	90	↓	4.59E-4	-4.91	-2.59	7,733 (top 9%)	(66)

Table III. Continued.

Gene	Cancer	Subtype	Case no.	Change expression	P-value (cancer/ normal)	*t*-test (cancer/ normal)	Fold change (cancer/ normal)	% Gene ranking	Database reference
	Lymphoma	Nodular lymphocyte-predominant Hodgkin's lymphoma	67	↑	7.61E-5	10.55	2.65	821 (top 5%)	(89)
	Head-neck	Nasopharyngeal carcinoma	41	↑	5.08E-4	3.57	1.56	1,794 (top 10%)	(90)
	Ovarian	Ovarian endometrioid adenocarcinoma	50	↑	0.001	3.96	1.76	506 (top 3%)	(91)
	Testis	Testicular seminoma	30	↑	0.007	8.03	3.21	806 (top 6%)	(92)
	Prostate	Prostate carcinoma	21	↓	2.12E-4	-4.26	-1.91	279 (top 2%)	(93)
	Brain	Oligodendroglioma	42	↓	4.42E-4	-6.05	-1.73	689 (top 9%)	(58)
	Melanoma	Cutaneous melanoma	87	↓	6.22E-4	-4.70	-6.74	927 (top 5%)	(80)

determination and contributes to the maintenance of the adult salivary gland homeostasis (11,31). Our database analysis indicated that ASCL3 was highly expressed in invasive ductal breast carcinoma (2.26-fold change, P=0.002 and gene ranking in the top 2%) compared with normal tissues (Table IV). Of the 21 analyzed tumor types, 5 exhibited a correlation with downregulation of ASCL3 (Table IV).

Analysis of various renal tumor subtypes indicated that ASCL3 exhibited a lower expression in renal oncocytoma with a fold change of -1.60, P=5.15E-16 and gene ranking in the top 3%. ASCL3 expression is downregulated in cervical cancer with a fold change of -1.65, P=1.24E-9 and gene ranking in the top 1%. In superficial bladder cancer, we found that ASCL3 also exhibited a lower expression, with a fold change of -1.63, P=1.16E-7 and the gene ranking in the top 3%. In anaplastic large-cell lymphoma, ASCL3 exhibited lower expression, with a fold change of -1.96, P=1.58E-5 and the gene ranking in the top 8%. Melanomas and basal cell skin carcinoma (also referred to as basalioma, the most common malignant skin tumor), exhibited ASCL3 downregulation with a fold change of -1.80, P=0.007 and the gene ranking in the top 10% (Table IV). Thus, ASCL3 ranked in the top 2% of genes exhibiting upregulation in breast cancer, while in renal, cervical and bladder cancer, lymphoma and melanoma, ASCL3 displayed significant downregulation compared with normal tissues (Table IV). These findings indicated that ASCL3 may be differentially expressed in specific types of cancer and that further investigation is required to determine the mechanisms underlying the involvement of ASCL3 in tumorigenesis.

ASCL4. ASCL4 (HASH4, bHLHa44) expression is associated with skin development. ASCL4 exhibited a 7-fold higher expression in fetal skin compared with adult skin (12). The role of ASCL4 in cellular function remains elusive. Therefore, comparative genomic sequencing did not reveal any function for this gene (32). ASCL4 expression did not satisfy the selection criteria of the present study; therefore, it was not selected for further investigation.

ASCL5. We were unable to obtain any data regarding ASCL5 based on the literature search through the PubMed database. Analysis of the Gene Ontology database indicated that ASCL5 may be involved in the regulation of DNA-templated transcription (33). Our bioinformatics analysis suggested that ASCL5 was upregulated in lung cancer with a fold change of 1.96-3.71, P-values of 0.002-0.003 and the gene ranking 5-9%. However, ASCL5 was downregulated in the majority of types of brain tumors, such as glioblastoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma and oligodendroglioma, with a fold change of -3.10 to -5.50, P-values of 0.002-5.25E-12 and the gene ranking 1-6% (Table V). To the best of our knowledge, our bioinformatics analysis is the first report to provide any information regarding the potential role of ASCL5 in tumorigenesis.

ASCL family in clinical application. In this report, we presented an *in silico* analysis of the ASCL gene family and investigated the potential involvement of ASCL genes in various cancers. Our meta-analysis approach provided a conspectus of the clinical data related to the ASCL gene

Table IV. Achaete-scute complex-like (ASCL) 3 expression in cancer.

Gene	Cancer	Subtype	Case no.	Change expression	P-value (cancer/ normal)	*t*-test (cancer/ normal)	Fold change (cancer/ normal)	% Gene ranking	Database reference
ASCL3	Breast	Invasive ductal breast carcinoma	30	↑	0.002	3.33	2.26	220 (top 2%)	(94)
	Kidney	Renal oncocytoma	92	↓	5.15E-16	-14.47	-1.60	340 (top 3%)	(95)
		Clear cell renal cell carcinoma	20	↓	2.48E-4	-4.66	-3.08	1,143 (top 10%)	(95)
	Cervical	Cervical cancer	84	↓	1.24E-9	-8.49	-1.65	96 (top 1%)	(96)
	Bladder	Superficial bladder cancer	60	↓	1.16E-7	-6.62	-1.63	274 (top 3%)	(75)
	Lymphoma	Anaplastic large-cell lymphoma	60	↓	1.58E-5	-8.87	-1.96	1,547 (top 8%)	(97)
	Melanoma	Skin basal cell carcinoma	87	↓	0.007	-2.74	-1.80	1,915 (top 10%)	(80)

Table V. Achaete-scute complex-like (ASCL) 5 expression in cancer.

Gene	Cancer	Subtype	Case no.	Change expression	P-value (cancer/ normal)	*t*-test (cancer/ normal)	Fold change (cancer/ normal)	% Gene ranking	Database reference
ASCL5	Lung	Small-cell lung carcinoma	73	↑	0.003	4.91	3.71	442 (top 5%)	(60)
		Squamous cell lung carcinoma	73	↑	0.002	3.26	1.96	911 (top 9%)	(60)
	Brain	Glioblastoma	54	↓	5.25E-12	-11.49	-3.10	141 (top 1%)	(52)
		Anaplastic oligoastrocytoma	54	↓	1.57E-4	-7.74	-3.79	322 (top 3%)	(52)
		Anaplastic oligodendroglioma	54	↓	7.70E-4	-13.99	-5.50	480 (top 4%)	(52)
		Oligodendroglioma	54	↓	0.002	-5.74	-4.24	878 (top 6%)	(52)

family and suggested that alterations in the ASCL genes may result in development of various tumors. Moreover, our analysis utilized the integration and validation of numerous microarray datasets, thereby allowing the use of an ASCL gene with its correlated cancers and its subtypes as future biomarkers for future cancer studies.

It was previously indicated that ASCL1 functions as an oncogene in lung cancer (23). Recent findings also demonstrated that ASCL1 is a marker for small-cell lung carcinomas (23,34). These data are consistent with our bioinformatics analyses (Fig. 1). ASCL1 exhibited significant overexpression in half of the analyzed cancer types (10 of 20 cancers), with the gene ranking in the top 10%. Moreover, a significant number of tumors exhibited ASCL1 downregulation, with the gene ranking in the top 1% (Fig. 1). ASCL1 was in the top 1% ranking of all overexpressed genes in leukemia, brain and lung cancers. Interestingly, gastric cancers and melanoma displayed downregulation of ASCL1, with the gene ranking in the top 1% of all downregulated genes.

Previous studies have suggested that ASCL2 is strongly expressed in colon cancer tissues and cell lines (HT-29 and LS174T cells) and that selective blockade of ASCL2 results in the inhibition of xenograft tumor growth, proliferation, invasion and migration (10,29,30). ASCL2 may promote colorectal (30), lung (35) and gastric cancer (36), suggesting a crucial role for ASCL2 involvement in tumor development. These data are consistent with our bioinformatics analysis (Fig. 1). Strikingly, ASCL2 expression analysis indicated increased mRNA expression in some cancer tissues and decreased expression in others. ASCL2 is in the top 10% of genes exhibiting overexpression in breast, colorectal, lung, gastric, head-neck, ovarian and testicular cancers, as well as lymphoma. However, brain tumor and melanoma subtypes exhibited significant reductions in the expression of ASCL2 when compared with normal tissues (Table III). Of note, ASCL3 expression displayed a wide range of mRNA levels in various cancers. ASCL3 was in the top 2% of overexpressed genes in breast cancer. Conversely, in lymphomas, melanomas,

renal, cervical and bladder cancers, ASCL3 expression was significantly reduced compared with that in normal tissues (Table IV). Expression analysis of ASCL5 suggested a correlation between elevated ASCL5 expression and lung cancer development. ASCL5 was one of highly expressed genes, ranking 5-9% in lung cancer. Interestingly, ASCL5 was downregulated in most types of brain tumors, such as glioblastoma, anaplastic oligoastrocytoma and anaplastic oligodendroglioma. The decrease in fold change ranged from -3.10 to -5.50, the P-values ranged from 0.002 to 5.25E-12, with the gene ranking 1-6% (Table V). Intriguingly, ASCL members exhibited increased expression in some cancer tissues and decreased expression in others. This is particularly apparent for ASCL2, ASCL3 and ASCL5 that displayed mRNA expression changes (either up-or downregulated in specific cancers). According to these data, both the up- and downregulation of ASCL genes may play an important role in tumor development. The emerging view of the unique developmental niche of ASCL members in early progenitors of diverse neural lineages suggests a potentially critical role in injury response, wound healing and tumorigenesis. However, a limited number of studies to date suggest that these ASCL members may contribute significantly to cancer development. The available data collectively suggest that alterations in the expression of ASCL genes may affect cellular behavior, such as cell proliferation, thereby initiating tumor development. The present study demonstrated that ASCL members may be involved in tumor development and introduces ASCL genes as potential candidates for future prognostic and therapeutic targets.

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