Journal of Basic & Clinical Medicine

http://www.sspublications.org/index.php/JBCM/index

Cancer Stem Cell Theory and Intratumor Heterogeneity in Thyroid Carcinogenesis

Masahiro Nakashima[1*], Mika Shimamura[2], Kazuaki Yasui[2], Norisato Mitsutake[3], Mutsumi Matsuu-Matsuyama[1], Katsuya Matsuda[1], Yuji Nagayama[2]

[1]Department of Tumor and Diagnostic Pathology, [2]Department of Molecular Medicine, and [3]Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

Journal of Basic & Clinical Medicine 2015, 4(1):8-12

Abstract

Intratumor heterogeneity is becoming a main concern not only biologically but also clinically, because it is critical to determine the cancer cells that have the potential to contribute to disease progression and to develop more effective cancer therapies. Although intratumor heterogeneity is also well recognized in thyroid cancer, it remains to be elucidated regarding how the heterogeneity arises in a tumor. The cancer stem-like cells (CSCs) are considered to cause the heterogeneous lineages of cancer cells that comprise the tumor. The epidemiologic study among Japanese atomic-bomb survivors and our animal experiments indicate that the presence of tumor-initiating cells (TICs) starts only in childhood and persists for over 50 years in thyroid gland, and these cells should be a subpopulation of immature thyroid cells possessing a life long-lasting or self-renewal capability, suggesting their stem cell-like properties. Although several attempts have been performed to identify thyroid CSCs, there is no convincing evidence demonstrating the presence of thyroid CSCs. We hypothesize that there may be hierarchically different CSCs that possess variable levels of tumorigenic potential and exert their tumorigenic potential depending on individually suitable microenvironment in a thyroid tumor.

Keywords: Thyroid cancer, cancer stem cell, heterogeneity, ALDH, EMT

Introduction

Two theories have been proposed in thyroid follicular cell carcinogenesis: the fetal cell carcinogenesis theory and the more common multistep carcinogenesis theory (1-3). In fetal cell carcinogenesis theory, cancer is regarded as an abnormal development of fetal thyroid cells and genomic alternations play an oncogenic role by preventing thyroid fetal cells from differentiating. According to the multistep carcinogenesis theory, most cancers are clonal in origin, arising from a single abnormal cell and progressing as a result of accumulation of inheritable molecular alterations (2). This multistep carcinogenesis model was originally shown convincingly in colorectal carcinoma by Vogelstein et al. (3). However, the hypothesis of another tumorigenic pathway, that of *de novo* carcinogenesis, suggesting the development of a cancer from normal colonic mucosa, without the intervening step, has been a matter of discussion for decades (4, 5). Indeed, colorectal *de novo* cancers account for a considerable proportion in Japan and the expected probabilities of developing *de novo* cancer are 18.6% for men, and 27.4% for women with early cancer (6). In *de novo* cancer theory, tumor-initiating cells (TICs) should be phenotypically malignant. There is accumulating evidence that solid tumors, including colorectal cancer, contain a distinct subpopulation of cancer stem-like cells (CSCs) or TICs that play important roles in cancer initiation, progression, recurrence, and metastasis. Therefore, identification and analysis of CSCs are fundamental for cancer study. We review here regarding the recent evidence of the CSCs presence in thyroid cancer and discuss about the possible involvement of CSCs during thyroid carcinogenesis.

Heterogeneity of thyroid cancers

Thyroid cancers of follicular cell origin can be classified as well differentiated carcinoma (WDC), such as papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), poorly differentiated carcinoma (PDC), and anaplastic carcinoma (AC) (7). Although the overall prognosis of WDC is excellent, PDC is moderately aggressive and AC is one of the most malignant human cancers with a significantly poor outcome (8). Molecular analyses have revealed the involvement of three main alterations including *RET/PTC* rearrangements, $BRAF^{V600E}$, and *RAS* mutations in PTC, and two main alterations including *RAS* mutations and *PAX8/PPARγ* rearrangements in FTC (9-17). These molecular alterations are observed in a mutually exclusive manner and considered to contribute to the initiation of normal follicular cells during thyroid carcinogenesis. On the other hand, mutations of both *TP53* gene and *CTNNB1* gene encoding β-catenin are restricted to PDC and AC, suggesting their significance in the late stage of thyroid carcinogenesis. Thus, thyroid cancers of follicular cell origin are a heterogeneous entity on clinical, pathological, and genetic points of view.

Molecular dissection of the genome has clearly shown cancer heterogeneity within organ sites and within tumors (18, 19). Intratumor heterogeneity is well recognized also in thyroid cancers. For PTC, several studies with fluorescence *in situ* hybridization analysis for *RET/PTC* rearrangements revealed that only subpopulation of tumor cells exhibited this rearrangement in PTCs (20-22). Another study demonstrated overexpression of the genes encoding TGF-β and integrin pathway molecules in the invasive peripheral region compared to the central region of same

Accepted in January, 2015

*Correspondence author: Masahiro Nakashima, M.D., Ph.D.

Department of Tumor and Diagnostic Pathology, Atomic Bomb Disease Institute, Nagasaki University

1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Phone: +81-95-819-7105, Fax: +81-95-819-7108

E-mail: moemoe@nagasaki-u.ac.jp

tumor, while these genes are known to be involved in epithelial-to-mesenchymal transition (EMT), (23). Also, a study on the AC cases with different microscopic appearances (e.g., spindle or giant cell morphology) has found that mutations in the *CTNNB1* gene are common but focal in AC (24). Intratumor heterogeneity is becoming a main concern not only biologically but also clinically, because it is critical to determine the cancer cells that have the potential to contribute to disease progression and to develop more effective cancer therapies. However, it remains to be elucidated regarding how heterogeneity arises in a tumor during thyroid carcinogenesis.

Heterogeneity and CSC model

Three different mechanisms are considered to cause the intratumor heterogeneity including: i) stochastic clonal evolution through genetic or epigenetic changes, which involves intrinsic differences; ii) environmentally determined effects which confer phenotypic and functional differences upon cancer cells in different locations; and iii) CSC model (25, 26). CSC could be defined as a small subset of cancer cells that self-renew to generate additional CSC as well as to differentiate to phenotypically diverse cancer cells including nontumorigenic cells with limited proliferative potential, creating a hierarchical organization. The CSC model is based on the presence of such undifferentiated cells, which are primarily responsible for cancer growth and disease progression. The presence of only mature differentiated cells in residual tumor masses after chemotherapy is a favorable prognostic factor, while the presence of residual undifferentiated cells predicts disease recurrence (27). CSCs have been initially identified in acute myeloid leukemia (28). Compelling data support the CSC model in various human solid cancers including breast cancers, brain cancers, colon cancers, pancreatic cancers, and ovarian cancers (29-34), suggesting several cancers consist of phenotypically and functionally heterogeneous cancer cells. However, the thyroid CSC remains to be confirmed.

Evidence for the presence of TICs showing stem cell-like properties in thyroid

It is well established that exposure to ionizing radiation, especially during childhood or adolescence, is a risk factor for thyroid cancer. Japanese atomic-bomb (A-bomb) survivors have been a critical source of information for insight into radiation-related thyroid cancer risk. Recently, a long-lasting thyroid cancer risk was reported for A-bomb survivors who were exposed to bombing at age of younger than 20-year-old (35). The risk decreased sharply with increased age at exposure and there was little evidence of increased thyroid cancer rate for those exposed after age 20. This study estimated that the excess thyroid cancer risk associated with childhood exposure has persisted for over 50 years after exposure, suggesting that TICs only occur in childhood and persist for several decades. Furthermore, our animal experiments with local irradiation (8 Gy) to the anterior neck of rats confirmed the frequent occurrence of thyroid cancers in childhood (4- and 7-week-old) exposure group but not in adulthood (7-month-old) exposure group (Fig. 1). This study also found that there were no significant differences in the number of DNA double strand breaks of follicular cells at acute phase (within 24 hours) after irradiation between both groups (Fig. 2), indicating that a high dose irradiation can easily induce DNA damage in follicular cells in any age but no TICs in adulthood. Another study by us with transgenic mouse demonstrated that although the expression of $BRAF^{V600E}$ in all thyroid cells from the fetal could lead to tumorigenesis, the postnatal expression of $BRAF^{V600E}$ in a small population of thyroid cells is not able to induce thyroid cancer (36). We speculate that the cell-of-origin for TIC is restricted to a certain subpopulation of postnatal thyroid gland and the $BRAF^{V600E}$ is required to be specifically expressed in such cells to induce thyroid cancers. When considering the difference between young and adult thyroids, we should take into account the involvement of normal stem/progenitor cells for thyroid carcinogenesis. Taken together these findings, TICs should be a subpopulation of immature thyroid cells possessing a life long-lasting and/or self-renewal capability, suggesting their stem cell-like properties.

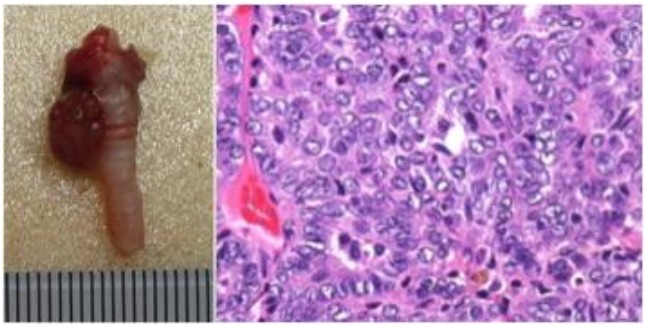

Figure 1: Macroscopic and histologic features of radiation-induced thyroid cancer at 16 months after exposure of a 7-week-old rat to 4 Gy local radiation.

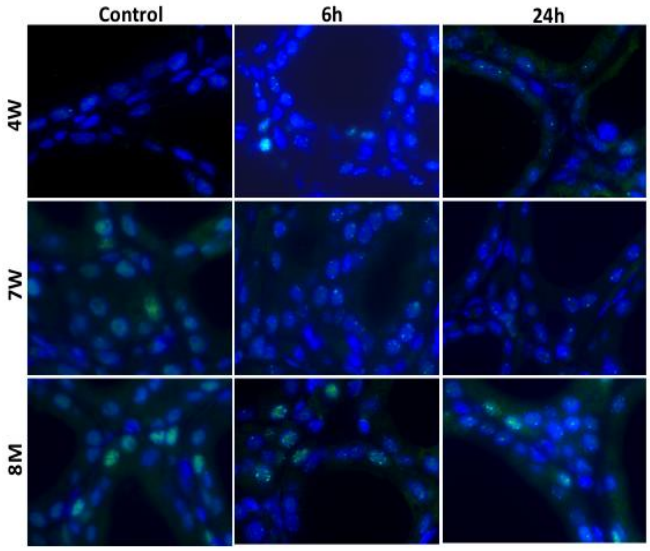

Figure 2: Alteration of the number of DNA double strand breaks in rat thyroid follicular cells after irradiation by immunofluorescence analysis of the 53BP1, a DNA damage response molecule. There are no significant differences in the number of 53BP1 nuclear foci at each time point (control, 6 hours, and 24 hours) after irradiation among 4-week-, 7-week-, and 8-month-old rats.

Studies to identify CSC in thyroid cancers

The "gold standard" to identify CSC is to isolate cells using biomarkers (e.g., cell surface marker) and inject them into immunocompromised mice to see whether they develop tumors (37). Regarding the biomarkers, recent studies have reported several CSC markers in solid tumors, e.g., $CD44^{+}/CD24^{-}$ for breast cancer (38), $CD133^{+}$ for brain tumor (30) and colon cancer (31),

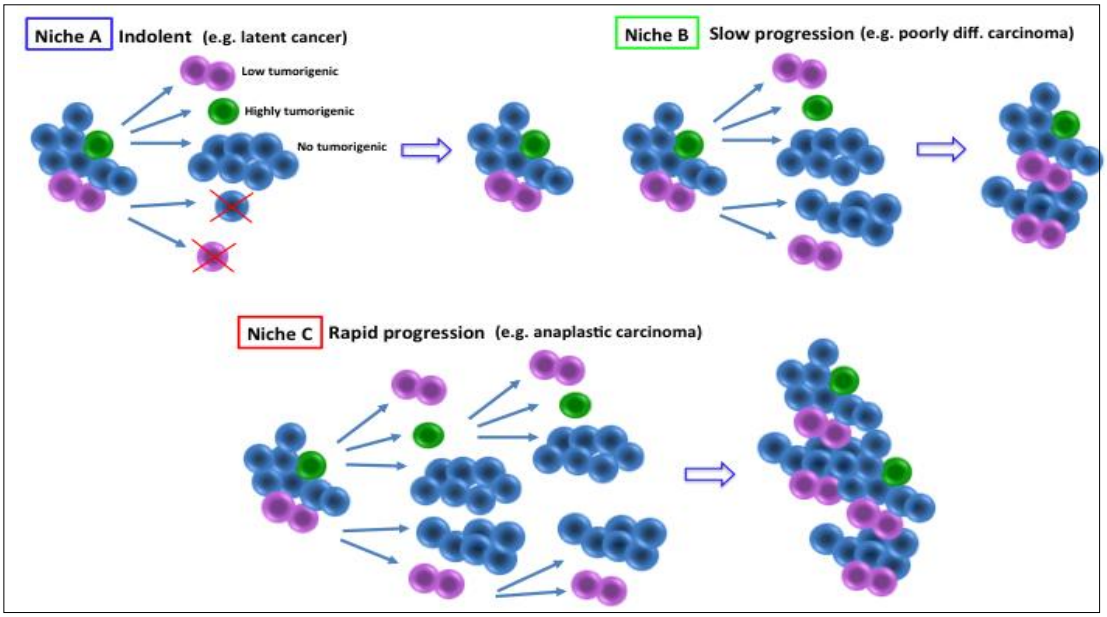

Figure 3: Hypothesis of a variable model to understand the range of disorders from indolent (e.g., latent cancer) to slowly (e.g., poorly differentiated carcinoma) or rapidly progressive (e.g., anaplastic carcinoma) and the intratumor heterogeneity. There may be hierarchically different CSCs, which possess variable levels of tumorigenic potential and exert their tumorigenic potential depending on individually suitable microenvironment (CSC niche) in a single tumor.

Table 1: Studies on identifying cancer stem-like cells in thyroid cancer

	CSC marker	Subjects	Self-renewal	In vivo tumor xenograft	Sphere colony assay
Mitsutake N Endocrinology (2007)	Side population	Non-thyroid cell line: ARO	Yes	Yes	Yes
		Thyroid cell line: FRO,TPC1,WRO	No data	No data	No data
Zito G PLoS one (2008)	CD133	Non-thyroid cell line: ARO, KAT-4	Yes	No data	Yes
		Thyroid cell line: KAT-18, FRO	No data	No data	No data
Friedman S PLoS one (2009)	CD133	Non-thyroid cell line: ARO	Yes	Yes	No data
		Thyroid cell line: FRO, TPC	No data	No data	No data
Todaro M Cancer Res (2010)	ALDH CD133 CD44	Primary thyroid cancer	Yes	Yes	Yes
Shimamura M Endocrine J (2013)	ALDH CD326	Thyroid cell line: FRO, KTC3, ACT1, 8505C	Yes	Yes	Yes

and $CD44^+$ for gastric cancer (39). In thyroid cancer, as shown in Table 1, side population (40) and $CD133^+$ (41, 42) are originally reported as CSC markers by using ARO and KAT-4 cells. However, these two cell lines are now thought to be identical to the colon cancer cell line, HT-29 (43). Recently, Todaro et al. demonstrated that a small subpopulation with high aldehyde dehydrogenase (ALDH) activity showed CSC-like features/tumorigenic ability by using primary thyroid cancer cells (44). Furthermore, Shimamura et al. reported that three (FRO, KTC3, and ACT1) out of the four AC-derived cell lines with a high ALDH activity showed CSC-like features, while none of the WDC-derived cell lines including the KTC1, TPC, and WRO, did not show SC-like features regardless of the ALDH activity (45). This study also evaluated whether ALDH-high fraction exhibited a symmetric division, a hallmark of CSC. As the results, ALDH-high fraction possessed both self-renewal and differentiation capacities generating both same and different heterogeneous populations, however, ALDH-low fraction also gave rise to both two populations. Thus, these subpopulations did not follow a classical hierarchical model. ALDH activity is probably a major candidate marker to enrich thyroid CSCs but not universal. Furthermore,

Yasui et al. demonstrated that the ACT1 (an AC-derived cell line) exhibited EMT phenotype; the number of ALDH-positive fraction decreased; and the ALDH-negative fraction gained greater tumor-forming ability than ALDH-positive fraction by induction of SNAIL expression (46). EMT is thought to play a critical role in the invasion and metastasis of cancer and to be associated with CSC properties (47, 48). Given that expression of SNAIL is restricted at the invasive fronts of thyroid cancers with an EMT phenotype (23), the role for ALDH in CSC properties may be dependent on the EMT status. In thyroid cancer, therefore, the state of CSC might be also flexible in certain environments. There may be different CSC markers depending on individual cases. Further characterization is needed to understand the whole view of thyroid CSCs.

Summary

A model of cancer progression that is more suited to the current understanding of cancer biology is one of variable progression depending on tumor type and stromal microenvironment (49). In thyroid carcinogenesis, a variable model should be more suitable to understand the range of disorders from indolent (e.g., latent cancer, papillary microcarcinoma, and conventional WDC) to slowly (e.g., aggressive variants of WDC and PDC) or rapidly progressive (e.g., AC) and the intratumor heterogeneity (50). We hypothesize that there may be hierarchically different CSCs that possess variable levels of tumorigenic potential and exert their tumorigenic potential depending on individually suitable microenvironment (CSC niche) even in a single tumor (Fig. 3). For instance, latent cancer or papillary microcarcinoma may be mainly consistent with no/low tumorigenic cells in the indolent niche, while AC may be arisen when high tumorigenic CSCs activate under the rapidly progressive niche. Indeed, our studies suggest that high tumorigenic CSCs are isolated only from AC-derived cell lines but not from WDC-derived cell lines, and their tumorigenic potential appears to be influenced by acquisition of EMT characteristics, which are strongly associated with circumstances in a single tumor (45, 46). Although several attempts have been performed to date to identify thyroid CSCs, there is no convincing evidence demonstrating the presence of thyroid CSCs. Studies on thyroid CSCs are still limited and further researches are definitely required.

Acknowledgement

We are grateful to Professor Kennichi Kakudo for his invitation to this special supplement on thyroid carcinogenesis.

References

1. Takano T. Fetal cell carcinogenesis of the thyroid: a hypothesis for better understanding of gene expression profile and genomic alternation in thyroid carcinoma. Endocr J 2004; 51:509-15.
2. Nowell PC. The clonal evolution of tumor cell populations. Science 1976; 194:23-8.
3. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988; 319:525-32.
4. Kuramoto S, Oohara T. Minute cancers arising de novo in the human large intestine. Cancer 1988; 61:829-34.
5. Shimoda T, Ikegami M, Fujisaki J, Matsui T, Aizawa S, Ishikawa E. Early colorectal carcinoma with special reference to its development de novo. Cancer 1989; 64:1138-46.
6. Goto H, Oda Y, Murakami Y, Tanaka T, Hasuda K, Goto S, Sasaki Y, Sakisaka S, Hattori M. Proportion of de novo cancers among colorectal cancers in Japan. Gastroenterology 2006; 131:40-6.
7. DeLellis RA, Lloyd RV, Heitz PU and Eng C. Tumours of Endocrine Organs, World Health Organization Classification of Tumours; Pathology and Genetics. Lyon: IARC Press; 2004.
8. Sipos JA, Mazzaferri EL. Thyroid cancer epidemiology and prognostic variables. Clin Oncol (R Coll Radiol) 2010; 22:395-404.
9. Jhiang SM, Mazzaferri EL. The ret/PTC oncogene in papillary thyroid carcinoma. J Lab Clin Med 1994; 123:331-7.
10. Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI, Ohtsuru A, Saenko VA, Kanematsu T, Yamashita S. Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. J Clin Endocrinol Metab 2003; 88:4393-7.
11. Suarez HG, Du Villard JA, Caillou B, Schlumberger M, Tubiana M, Parmentier C, Monier R. Detection of activated ras oncogenes in human thyroid carcinomas. Oncogene 1988; 2:403-6.
12. Namba H, Gutman RA, Matsuo K, Alvarez A, Fagin JA. H-ras protooncogene mutations in human thyroid neoplasms. J Clin Endocrinol Metab 1990; 71:223-9.
13. Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, Nikiforov YE. PAX8–PPAR gamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. Am J Surg Pathol 2002; 26:1016-23.
14. Marques AR, Espadinha C, Catarino AL, Moniz S, Pereira T, Sobrinho LG, Leite V. Expression of PAX8–PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas. J Clin Endocrinol Metab 2002; 87:3947-52.
15. Suárez HG, Du Villard JA, Caillou B, Schlumberger M, Tubiana M, Parmentier C, Monier R. Detection of activated ras oncogenes in human thyroid carcinomas. Oncogene 1988; 2:403-6.
16. Lemoine NR, Mayall ES, Wyllie ES, Williams ED, Goyns M, Stringer B, Wynford-Thomas D. High frequency of ras oncogene activation in all stage of human thyroid tumorigenesis. Oncogene 1989; 4:159-64.
17. Namba H, Rubin SA, Faqin JA. Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol 1990; 4:1474-9.
18. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012; 366:883-92.
19. Shibata D. Cancer. Heterogeneity and tumor history. Science 2012; 336:304-5.
20. Unger K1, Zitzelsberger H, Salvatore G, Santoro M, Bogdanova T, Braselmann H, Kastner P, Zurnadzhy L, Tronko N, Hutzler P, Thomas G. Heterogeneity in the distribution of RET/PTC rearrangements within individual post-Chernobyl papillary thyroid carcinomas. J Clin Endocrinol Metab 2004; 89:4272-9.
21. Zhu Z, Ciampi R, Nikiforova MN, Gandhi M, Nikiforov YE.

Prevalence of RET/PTC rearrangements in thyroid papillary carcinomas: effects of the detection methods and genetic heterogeneity. J Clin Endocrinol Metab 2006; 91:3603-10.
22. Hieber L1, Huber R, Bauer V, Schäffner Q, Braselmann H, Thomas G, Bogdanova T, Zitzelsberger H. Chromosomal rearrangements in post-Chernobyl papillary thyroid carcinomas: evaluation by spectral karyotyping and automated interphase FISH. J Biomed Biotechnol 2011; 2011:693691.
23. Vasko V, Esinosa AV, Scouten W, He H, Auer H, Liyanarachchi S, Larin A, Savchenko V, Francis GL, de la Chapelle A, Saji M, Ringel MD. Gene expression and functional evidence of epithelial-to-mesenchymal transition in papillary thyroid carcinoma invasion. Proc Natl Acad Sci USA 2007; 104:2803-8.
24. Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL. Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. Cancer Res 1999; 59:1811-5.
25. Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. Cancer Cell 2012; 21:283-96.
26. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature 501; 2013:328-37.
27. Stenning SP, Parkinson MC, Fisher C, Mead GM, Cook PA, Fossa SD, Horwich A, Jones WG, Newlands ES, Oliver RT, Stenwig AE, Wilkinson PM. Postchemotherapy residual masses in germ cell tumor patients: content, clinical features, and prognosis. Medical Research Council Testicular Tumour Working Party. Cancer 1998; 83:1409-19.
28. Blair A, Hogge DE, Ailles LE, Lansdorp PM, Sutherland HJ. Lack of expression of thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. Blood 1997; 89:3104-12.
29. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003; 100:3983-8.
30. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003; 63:5821-8.
31. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007; 445:106-10.
32. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. Cancer Res 2007; 67:1030-7.
33. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, Mor G. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 2009; 8:158-66.
34. Curley MD, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, Roberts DJ, Seiden MV, Scadden DT, Rueda BR, Foster R. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells 2009; 27:2875-83.
35. Furukawa K1, Preston D, Funamoto S, Yonehara S, Ito M, Tokuoka S, Sugiyama H, Soda M, Ozasa K, Mabuchi K. Long-term trend of thyroid cancer risk among Japanese atomic-bomb survivors: 60 years after exposure. Int J Cancer. 2013; 132:1222-6.
36. Shimamura M, Nakahara M, Orim F, Kurashige T, Mitsutake N, Nakashima M, Kondo S, Yamada M, Taguchi R, Kimura S, Nagayama Y. Postnatal expression of BRAFV600E does not induce thyroid cancer in mouse models of thyroid papillary carcinoma. Endocrinology 2013; 154:4423-30.
37. Maenhaut C, Dumont JE, Roger PP, van Staveren WC. Cancer stem cells: a reality, a myth, a fuzzy concept or a misnomer? An analysis. Carcinogenesis 2010; 31:149-58.
38. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1:555-67.
39. Takaishi S, Okumura T, Tu S, Wang SS, Shibata W, Vigneshwaran R, Gordon SA, Shimada Y, Wang TC. Identification of gastric cancer stem cells using the cell surface marker CD44. Stem Cells 2009; 27:1006-20.
40. Mitsutake N, Iwao A, Nagai K, Namba H, Ohtsuru A, Saenko V, Yamashita S. Characterization of side population in thyroid cancer cell lines: cancer stem-like cells are enriched partly but not exclusively. Endocrinology 2007; 148:1797-803.
41. Zito G, Richiusa P, Bommarito A, Carissimi E, Russo L, Coppola A, Zerilli M, Rodolico V, Criscimanna A, Amato M, Pizzolanti G, Galluzzo A, Giordano C. *In vitro* identification and characterization of CD133(pos) cancer stem-like cells in anaplastic thyroid carcinoma cell lines. PLoS One 2008; 3:e3544.
42. Friedman S, Lu M, Schultz A, Thomas D, Lin RY. $CD133^{+}$ anaplastic thyroid cancer cells initiate tumors in immunodeficient mice and are regulated by thyrotropin. PLoS One 2009; 4:e5395.
43. Schweppe RE, Klopper JP, Korch C, Pugazhenthi U, Benezra M, Knauf JA, Fagin JA, Marlow LA, Copland JA, Smallridge RC, Haugen BR. Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification. J Clin Endocrinol Metab 2008; 93:4331-41.
44. Todaro M, Iovino F, Eterno V, Cammareri P, Gambara G, Espina V, Gulotta G, Dieli F, Giordano S, De Maria R, Stassi G. Tumorigenic and metastatic activity of human thyroid cancer stem cells. Cancer Res 2010; 70:8874-85.
45. Shimamura M, Nagayama Y, Matsuse M, Yamashita S, Mitsutake N. Analysis of multiple markers for cancer stem-like cells in human thyroid carcinoma cell lines. Endocrine J 2014; 61:481-90.
46. Yasui K, Shimamura M, Mitsutake N, Nagayama Y. SNAIL induces epithelial-to- mesenchymal transition and cancer stem cell–like properties in aldehyde dehydroghenase-negative thyroid cancer cells. Thyroid 2013; 23:989-96.
47. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M. The epithelial–mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133:704-15.
48. Kong D, Banerjee S, Ahmad A, Li Y, Wang Z, Sethi S, Sarkar FH. Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. PLoS One 2010; 5:e12445.
49. Esserman LJ, Thompson IM, Reid B, Nelson P, Ransohoff DF, Welch HG, Hwang S, Berry DA, Kinzler KW, Black WC, Bissell M, Parners H, Srivastava S. Addressing overdiagnosis and overtreatment in cancer: a prescription for change. Lancet Oncol 2014; 15:e234-42.
50. Kakudo K, Wakasa T, Ohta Y, Yane K, Satoh S, Yokoi T, Yamashita H. Moderate-risk and high-risk thyroid carcinomas of follicular cell origin. J Basic Clin Med 2014; 3:12-7.