New insights in optimizing treatment and the role of cancer stem cells in esophageal cancer

Honing, Judith

Chapter 1

General introduction

In the Netherlands the overall incidence of cancer is still increasing with approximately 40% in the last decade (1). Although technical and therapeutic options are expanding, the overall 5-year survival rate in curative treatable patients has increased with only 9%, from 47% to 56% (2), making cancer a huge burden on patients and society. In alignment with these numbers, the incidence of esophageal cancer has also increased steadily over the last decade from around 1900 patients per year to a current number of around 2500 esophageal cancer patients (3,4). These numbers are equal to other countries in the Western world (5,6). The majority of patients have the histological subtype esophageal adenocarcinoma (EAC), while esophageal squamous cell carcinoma (ESCC) more often occurs in Asian countries (5,7,8). In both types chronic irritation and inflammation seem to be important inducers of malignant transformation. In ESCC, alcohol consumption and tobacco use are the two main known risk factors (9,10), while in EAC, gastro-esophageal reflux disease (GERD) is the major risk factor (11). Other risk factors for EAC are sex, race and obesity, as EAC commonly occurs in white men (7,12), and is associated with an increased Body Mass Index (13). GERD is also known to be an important contributor to the development of Barrett's esophagus (BE), the precursor lesion of EAC (14). BE is a metaplastic lesion in which the normal squamous cell esophageal epithelium is partially replaced by a columnar epithelium which may progress to dysplasia and eventually EAC. Despite being a known precursor lesion, patients diagnosed with BE have a low risk of progressing towards EAC; per year, rates of 0.12-0.85% have been reported (15-17). Patients with BE receive a regular endoscopy scheme, as today we have no other tools yet to predict which patient will progress towards cancer.

After being diagnosed with esophageal cancer, patients are staged according to the current TNM 7th classification using endoscopic ultrasound (EUS), Computed Tomography (CT) scan and an 18F-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) or PET-CT scan (18). Standard treatment with curative intent consists of neoadjuvant chemoradiation (CRT) followed by a transthoracic esophagectomy (19). The Dutch CROSS trial showed a significant benefit of adding neoadjuvant CRT, consisting of carboplatin/paclitaxel with 41.4Gy, to surgery increasing the median overall survival up to 49.4 months compared to 24.0 months in patients with surgery alone (20). In 29% of patients treated with neoadjuvant CRT a pathologic complete response (pCR) was achieved, but also 18% showed little or no response to CRT. A complete response to neoadjuvant CRT is associated with a better survival in EC patients (21), but it is also known that even pathological complete responders may relapse early within one year, raising questions about

which factors predict these more aggressive tumors.

In patients who are not eligible for surgery, for instance due to tumors which are closely related with- or tethered to vital structures or due to severe comorbidity, definitive chemoradiation (dCRT) or radiotherapy (dRT) is the recommended standard curative treatment (22).

The main causes of poor prognosis of esophageal cancer patients are the tumor extent due to a late presentation of disease, early dissemination and a high recurrence rate. Therefore we need a better understanding of esophageal carcinogenesis and more insight in what drives its progression and recurrence. The Cancer Stem Cell (CSC) model could be helpful in achieving a better understanding of esophageal oncogenesis, which can be important to improve current therapy and to identify novel prognostic markers. This model proposes architecture of the tumor in which a subgroup of tumor cells has stem cell (SC) characteristics, particularly self-renewal and multilineage differentiation capacity, and has enhanced tumorigenic ability compared to the bulk tumor cells (23). The first study was done in leukaemia where CD34+/CD38- tumor cells derived from acute myeloid leukaemia patients had SC characteristics (24). In solid cancers, studies on CSCs have been done in various tumor types, including glioblastoma, breast and colon cancer (25-27).

CSCs are associated with high tumorigenicity and chemoresistance and could provide a possible explanation for current treatment failure and disease relapse. An important aspect of CSCs is their ability to escape cell death induced by conventional chemo- or radiotherapy for example by multidrug resistance due to up-regulation of cellular efflux pumps, quiescence and/or enhanced activation of DNA damage repair (28-30). Similar to normal SCs, CSCs are believed to have more effective DNA damage repair mechanisms that results in resistance to DNA-damaging treatments such as chemo-and radiotherapy. Evidence for this notion was provided in glioblastoma, where CSCs marked by CD133 were shown to repair DNA damage more actively by activating the DNA damage checkpoint (31). Another feature of CSCs is multidrug resistance as a result of the presence of ATP binding cassette (ABC) drug transporters. ABC drug transporters are higher expressed and active in SCs and CSCs, and this characteristic is used in the Hoechst efflux assay in which these cells can be identified as a population with low Hoechst intensity, known as the side population (32). Quiescence and slow cell division is considered a third feature of how CSCs can escape chemotherapeutics, since therapy-induced DNA damage and cell death manifests mostly during mitosis (30,33).

Besides resistance against current therapeutic options, CSCs have also been implicated in metastasis and the subsequent development of secondary tumors, possibly through epithelial to mesenchymal transition (EMT). EMT is a process where tumor cells can acquire a more mesenchymal state, which facilitates tumor cell migration and invasion and metastasis (34). In the epithelial state, cells express epithelial markers such as the membrane bound cell adhesion protein E-cadherin. During the conversion to a more mesenchymal state cells lose E-cadherin and gain expression of mesenchymal markers such as vimentin and fibronectin. Migrating cancer stem cells (MCSCs) have been proposed to have both features of a stemness and EMT phenotype and this subpopulation may be a driving factor in the development of metastases (35-37). According to this concept, MCSCs reside on the invasive front of the tumor and through EMT can disseminate from the tumor. As they have stemness features these migrating tumor cells could populate distant sites where they eventually form metastatic colonies. When taking together the above mentioned highly malignant properties of CSCs, it is easy to imagine that therapies that are effective in reducing the tumor load in patients, but fail to eradicate the CSCs fraction, will not be effective and result in relapse of disease.

Currently there is a strong interest in developing targeted agents against pathways that drive CSCs. For example, the Wnt and Hedgehog (Hh) pathways were found to be important in the maintenance of CSCs in various tumor types, including gastro-intestinal cancers(30). Essential in the canonical Wnt-pathway is β-catenin, which in the inactivated state is degraded by a complex consisting of glycogen synthase kinase 3β (GSK3β), Axin1 and Axin2, casein kinase 1 (CK1) and Adenoma Polyposis Coli (APC). Wnt ligands that activate Frizzled receptors cause the disruption of this degradation complex, leading to β-catenin accumulation in the cytoplasm and subsequent translocation to the nucleus. In the nucleus β-catenin binds to TCF/LEF transcription factors resulting in the transcriptional activation of specific target genes. The Wnt pathway has been implicated in normal SCs and homeostasis of various tissues, including the intestine and skin (38,39). The Wnt-pathway is also known to be involved in EMT (40) and therefore might have an important role in MCSCs (41). In colon cancer the Wnt pathway is known to play a central role, and CSCs could be identified using markers indicative for activity of this pathway (26). Lgr5 and CD44, both targets of the Wnt pathway could mark CSCs in intestinal and colon cancer (42,43).

The Hh pathway has been implicated in CSCs and Hh inhibition led to a reduction of CSCs in various tumors (44,45). The Hh pathway is silenced via the repression of

the signal transducer smoothened (Smo) by the transmembrane receptor Patched (Ptch). When one of the Hh ligands (Desert, Indian or Sonic) binds to Ptch, Smo repression is released leading to downstream activation of the transcription factor Gli-1, which regulates the expression of Hh target genes.

The identification of CSCs is essentially based on their ability to self-renew and to differentiate, allowing CSCs to repeatedly initiate tumors that resemble the cellular heterogeneity of the primary tumor. The use of mouse models in combination with lineage tracing experiments has proven to be effective for the identification of SCs and SC markers, which could be subsequently tested for their ability to identify CSCs in tumor models (43). Another frequently used approach to identify CSCs is the use of cell surface markers in order to isolate subpopulations of cells from patient tumor material or cell lines by FACS (46,47). The sorted cell fractions can be subsequently tested for their tumor forming potential using limited dilution and serial transplantation assays in immune-compromised mice. An alternative approach to study cells with CSC characteristics may be provided by the use of serum-free medium cell culturing known to lead to dedifferention of cells and acquisition of CSC characteristics (48,49). These cells often form a three-dimensional structure, called a spheroid, which has been shown to enrich for cells with SC characteristics (50,51). Other approaches to identify CSCs use specific SC properties such as high activity of specific enzymes or chemoresistance (52,53). For example, as mentioned above, the Hoechst exclusion assay is based the enhanced activity of multidrug resistance pumps, which efflux the dye Hoechst 33342 more efficiently in SCs/CSCs. High efflux cells can be identified by FACS analyses as a typical tail, also called the Side Population (SP). SP cells showed to be more tumorigenic in mice than non-SP (32). Likewise, Aldehyde dehydrogenase (ALDH1) is an enzyme known to be highly active in cells with CSC characteristics (54). The enzyme is important for the conversion of retinol in retinoic acid (RA), RA being a major regulator of differentiation by influencing the expression of specific sets of genes. Besides selecting for CSC properties, ALDH1 positive cells also have invasive and migratory capacities (55). Assuming that a higher proportion of CSCs in a tumor correlates with poor outcome, indirect validation of potential CSC markers has been investigated by examining their expression in tumor material in relation to recurrence and survival in patient cohorts. A limitation of such studies is that cells expressing these markers are not necessarily CSCs, since only functional testing can reveal their CSC characteristics.

In EC several approaches have been used to identify CSCs, however, with little success thus far. For example, the cell surface glycoprotein and Wnt-target gene

CD44 is associated with CSCs in various solid malignancies (42,48). High expression of CD44 has been found in ESCC and EAC cell lines with CSC characteristics, such as enhanced colony formation, radiotherapy resistance and increased tumor growth in vivo (56,57). Grotenhuis et al. detected tumor-initiating cells at low frequencies of around 1:64.000 cells in patient-derived EAC samples in in vivo serial transplantation assays, however, these CSCs could not be enriched using a panel of presumed CSC markers, including CD44 and CD24 (47). The expression of several known SC or CSC markers, such as ALDH1, BMI1 and SOX2, has been determined in esophageal cancer patients' cohorts to investigate their relation with survival and recurrence, but often in a limited number of patients or only in ESCCs patients (58-60). Because EAC is the most common histological subtype in Western countries more insights in the value of possible CSC markers in EAC is important.

A better understanding of esophageal carcinogenesis and more insight in the molecular mechanisms that drive disease progression is important to optimize current treatment modalities and to develop novel targeted therapeutics. In this respect, the possible role of CSCs in esophageal cancer is of interest. Therefore, the aim of this thesis was to improve the outcome of esophageal cancer patients by optimizing current treatment modalities and investigating possible new prognostic markers. In addition the role of CSCs in esophageal cancer using a cell line based spheroid model was investigated.

Outline of the thesis

Current standard treatment for esophageal cancer is a trimodality treatment consisting of neoadjuvant chemoradiotherapy (CRT) and surgery. After intended curative resection, histological examination of the resected specimen by an experienced pathologist is important to determine the microscopic radicality (R0) of the resection. Microscopic involvement of the longitudinal resection margins (R1) is a known independent prognostic factor for a poor survival. Involvement of the circumferential margin (CRM) also has been shown to be an important independent prognostic factor in esophageal cancer (61). However, the definite cut-off point has not been determined yet. In **chapter 2** we investigated the role of the CRM margin on prognosis and determined the optimal cut-off point in a relatively large patient group.

In the treatment of esophageal cancer patients who are not eligible for surgery, either due to technical irresectability or relative high comorbidity, definitive chemoradiotherapy (dCRT) is the preferred option. The two most often used chemotherapeutic regimens are: cisplatinum/ 5-fluorouracil (5-FU) and carboplatin/

paclitaxel. Although current guidelines advise cisplatinum/ 5-FU as the treatment of choice (22,62), no prospective randomized study yet compared the difference in survival outcome and toxicity profiles of both dCRT regimens. Carboplatin/paclitaxel is also often used in the neoadjuvant setting and is suggested to have a favourable toxicity profile (63). Therefore, in **chapter 3** we retrospectively analyzed survival outcome and toxicity rates between the two dCRT regimens in esophageal cancer patients treated in five centres in the Northeast Netherlands.

In esophageal cancer the CSC model remains yet to be confirmed. In EAC and ESCC several markers have been associated with tumor cells with CSC characteristics (56,64,65), however not consistently and a clear signature has not yet been defined. In **chapter 4** we investigated the potential of two spheroid cultured EAC cell lines to enrich for CSCs using in vitro and in vivo assays, and to identify pathways associated with CSCs by transcriptional profiling.

Currently, no prognostic markers in BE are known that indicate progression to malignancy. GATA6 is a transcription factor associated with several gastrointestinal malignancies and suggested to have an important role in activating the Wnt pathway in pancreatic cancer (66,67). GATA6 gene amplification also has been associated with the progression of BE towards EAC and has been implicated as a prognostic factor for EAC (68,69). However, the expression of the GATA6 protein during the various stages of progression towards EAC and its relation with survival outcome in EAC patients is not known. Therefore, in **chapter 5** the expression of GATA6 in all stages of BE to EAC development and its relation with survival outcome in EAC patients was further explored

In chapter 6 we hypothesized that if CSCs are responsible for tumor recurrence, presumed CSC markers might predict survival outcome in EAC patients and could provide new prognostic markers. Several proteins have been implicated to mark CSCs in gastrointestinal cancers. Axin2 and CD44, both targets of the Wnt pathway have been associated with CSCs in colon cancer (42,70). ALDH1, Bmi1 and SOX2 are proposed CSC markers in several cancers, including intestinal and pancreatic cancer (54,71,72), but their expression in relation to patient outcome has not been investigated as yet in EAC. **Chapter 6**, describes the expression of some of these presumed CSC markers, including ALDH1, Axin2, Bmi1, CD44 and SOX2 that were determined in EAC patients treated with surgery alone, and the expression rate was related to clinicopathological features and survival outcome.

With current neoadjuvant CRT followed by standard surgical procedures, patients can be divided in two clinically relevant pathologic subcategories of responders versus non-responders. This raises questions about the necessity of surgery in those patients with pathologic complete response, if we can identify factors that can predict response to therapy and survival outcome. Several markers, such as Sonic-Hedgehog (SHH), have been proposed as predictive marker for neoadjuvant CRT in esophageal cancer patients (73). In **chapter 7** the expression CD44, SHH and SOX2 was investigated in a cohort of esophageal cancer patients treated with neoadjuvant CRT.

REFERENCES

(1) Dutch Cancer Registration. Incidence of cancer in the Netherlands. 2011; Available at: www.cijfersoverkanker.nl/selecties/Dataset_1/img530b4f63bdc5d. Accessed 02/24, 2014.
(2) Dutch Cancer Registration. Survival all cancer patients in the Netherlands. 2005; Available at: www.cijfersoverkanker.nl/selecties/Dataset_2/img530b509261230. Accessed 02/24, 2014.
(3) Dutch Cancer Registration. Incidence of esophageal cancer in the Netherlands. 2012; Available at: http://cijfersoverkanker.nl/selecties/dataset_3/img535b85232a17e. Accessed 26/04/2014, 2014.
(4) Crane LM, Schaapveld M, Visser O, Louwman MW, Plukker JT, van Dam GM. Oesophageal cancer in The Netherlands: increasing incidence and mortality but improving survival. Eur J Cancer 2007 Jun;43(9):1445-1451.
(5) Castro C, Bosetti C, Malvezzi M, Bertuccio P, Levi F, Negri E, et al. Patterns and trends in esophageal cancer mortality and incidence in Europe (1980-2011) and predictions to 2015. Ann Oncol 2014 Jan;25(1):283-290.
(6) Kroep S, Lansdorp-Vogelaar I, Rubenstein JH, Lemmens VE, van Heijningen EB, Aragones N, et al. Comparing Trends in Esophageal Adenocarcinoma Incidence and Lifestyle Factors Between the United States, Spain, and The Netherlands. Am J Gastroenterol 2013 Dec 17.
(7) Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. J Natl Cancer Inst 2008 Aug 20;100(16):1184-1187.
(8) Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006 May 10;24(14):2137-2150.
(9) Pandeya N, Williams G, Green AC, Webb PM, Whiteman DC, Australian Cancer Study. Alcohol consumption and the risks of adenocarcinoma and squamous cell carcinoma of the esophagus. Gastroenterology 2009 Apr;136(4):1215-24, e1-2.
(10) Lubin JH, Cook MB, Pandeya N, Vaughan TL, Abnet CC, Giffen C, et al. The importance of exposure rate on odds ratios by cigarette smoking and alcohol consumption for esophageal adenocarcinoma and squamous cell carcinoma in the Barrett's Esophagus and Esophageal Adenocarcinoma Consortium. Cancer Epidemiol 2012 Jun;36(3):306-316.
(11) Rubenstein JH, Taylor JB. Meta-analysis: the association of oesophageal adenocarcinoma with symptoms of gastro-oesophageal reflux. Aliment Pharmacol Ther 2010 Nov;32(10):1222-1227.
(12) Lagergren J, Lagergren P. Recent developments in esophageal adenocarcinoma. CA Cancer J Clin 2013 Jul-Aug;63(4):232-248.
(13) Turati F, Tramacere I, La Vecchia C, Negri E. A meta-analysis of body mass index and esophageal and gastric cardia adenocarcinoma. Ann Oncol 2013 Mar;24(3):609-617.
(14) Fass R, Hell RW, Garewal HS, Martinez P, Pulliam G, Wendel C, et al. Correlation of oesophageal acid exposure with Barrett's oesophagus length. Gut 2001 Mar;48(3):310-313.
(15) Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011 Jul 6;103(13):1049-1057.
(16) Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011 Oct 13;365(15):1375-1383.
(17) Picardo SL, O'Brien MP, Feighery R, O'Toole D, Ravi N, O'Farrell NJ, et al. A Barrett's esophagus registry of over 1000 patients from a specialist center highlights greater risk of progression than population-based registries and high risk of low grade dysplasia. Dis Esophagus 2014 Jan 15.
(18) National Comprehensive Cancer Network (NCCN) Guidelines Esophageal and Esophagogastric

Junction Cancers. Version 2.2013 ESOPH-1; Available at: http://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf. Accessed 03/05, 2014.
(19) National Comprehensive Cancer Network (NCCN) Guidelines Esophageal and Esophagogastric Junction Cancers. Version 2.2013 ESOPH-E 1; Available at: http://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf. Accessed 03/05, 2014.
(20) van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012 May 31;366(22):2074-2084.
(21) Smit JK, Guler S, Beukema JC, Mul VE, Burgerhof JG, Hospers GA, et al. Different recurrence pattern after neoadjuvant chemoradiotherapy compared to surgery alone in esophageal cancer patients. Ann Surg Oncol 2013 Nov;20(12):4008-4015.
(22) Stahl M, Budach W, Meyer HJ, Cervantes A, ESMO Guidelines Working Group. Esophageal cancer: Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010 May;21 Suppl 5:v46-9.
(23) Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. Cell Stem Cell 2012 Jun 14;10(6):717-728.
(24) Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997 Jul;3(7):730-737.
(25) Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003 Apr 1;100(7):3983-3988.
(26) Vermeulen L, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol 2010 May;12(5):468-476.
(27) Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature 2004 Nov 18;432(7015):396-401.
(28) Sampieri K, Fodde R. Cancer stem cells and metastasis. Semin Cancer Biol 2012 Jun;22(3):187-193.
(29) Maugeri-Sacca M, Vigneri P, De Maria R. Cancer stem cells and chemosensitivity. Clin Cancer Res 2011 Aug 1;17(15):4942-4947.
(30) Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells - what challenges do they pose? Nat Rev Drug Discov 2014 Jul 1;13(7):497-512.
(31) Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006 Dec 7;444(7120):756-760.
(32) Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 1996 Apr 1;183(4):1797-1806.
(33) Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 2012 Aug 23;488(7412):522-526.
(34) Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008 May 16;133(4):704-715.
(35) Siebzehnrubl FA, Silver DJ, Tugertimur B, Deleyrolle LP, Siebzehnrubl D, Sarkisian MR, et al. The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance. EMBO Mol Med 2013 Aug;5(8):1196-1212.
(36) Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. Nat Rev Cancer 2005 Sep;5(9):744-749.
(37) Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator

ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 2009 Dec;11(12):1487-1495.
(38) Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007 Oct 25;449(7165):1003-1007.
(39) Lim X, Tan SH, Koh WL, Chau RM, Yan KS, Kuo CJ, et al. Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. Science 2013 Dec 6;342(6163):1226-1230.
(40) Kim K, Lu Z, Hay ED. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. Cell Biol Int 2002;26(5):463-476.
(41) Fodde R, Brabletz T. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. Curr Opin Cell Biol 2007 Apr;19(2):150-158.
(42) Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A 2007 Jun 12;104(24):10158-10163.
(43) Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009 Jan 29;457(7229):608-611.
(44) Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 2007 Jan 23;17(2):165-172.
(45) Mimeault M, Batra SK. Altered gene products involved in the malignant reprogramming of cancer stem/progenitor cells and multitargeted therapies. Mol Aspects Med 2013 Aug 29.
(46) Kemper K, Prasetyanti PR, De Lau W, Rodermond H, Clevers H, Medema JP. Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. Stem Cells 2012 Nov;30(11):2378-2386.
(47) Grotenhuis BA, Dinjens WN, Wijnhoven BP, Sonneveld P, Sacchetti A, Franken PF, et al. Barrett's oesophageal adenocarcinoma encompasses tumour-initiating cells that do not express common cancer stem cell markers. J Pathol 2010 Aug;221(4):379-389.
(48) Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 2005 Jul 1;65(13):5506-5511.
(49) Vermeulen L, Todaro M, de Sousa Mello F, Sprick MR, Kemper K, Perez Alea M, et al. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. Proc Natl Acad Sci U S A 2008 Sep 9;105(36):13427-13432.
(50) Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992 Mar 27;255(5052):1707-1710.
(51) Morrison BJ, Steel JC, Morris JC. Sphere culture of murine lung cancer cell lines are enriched with cancer initiating cells. PLoS One 2012;7(11):e49752.
(52) Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res 2007 May 15;67(10):4827-4833.
(53) Zhao Y, Bao Q, Schwarz B, Zhao L, Mysliwietz J, Ellwart J, et al. Stem cell-like side populations in esophageal cancer: a source of chemotherapy resistance and metastases. Stem Cells Dev 2014 Jan 15;23(2):180-192.
(54) Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, et al. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PLoS One 2010 Apr 21;5(4):e10277.
(55) Charafe-Jauffret E, Ginestier C, Iovino F, Tarpin C, Diebel M, Esterni B, et al. Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clin Cancer Res 2010 Jan 1;16(1):45-55.

(56) Smit JK, Faber H, Niemantsverdriet M, Baanstra M, Bussink J, Hollema H, et al. Prediction of response to radiotherapy in the treatment of esophageal cancer using stem cell markers. Radiother Oncol 2013 Jun;107(3):434-441.
(57) Zhao JS, Li WJ, Ge D, Zhang PJ, Li JJ, Lu CL, et al. Tumor initiating cells in esophageal squamous cell carcinomas express high levels of CD44. PLoS One 2011;6(6):e21419.
(58) DiMaio MA, Kwok S, Montgomery KD, Lowe AW, Pai RK. Immunohistochemical panel for distinguishing esophageal adenocarcinoma from squamous cell carcinoma: a combination of p63, cytokeratin 5/6, MUC5AC, and anterior gradient homolog 2 allows optimal subtyping. Hum Pathol 2012 Nov;43(11):1799-1807.
(59) Minato T, Yamamoto Y, Seike J, Yoshida T, Yamai H, Takechi H, et al. Aldehyde dehydrogenase 1 expression is associated with poor prognosis in patients with esophageal squamous cell carcinoma. Ann Surg Oncol 2013 Jan;20(1):209-217.
(60) Yoshikawa R, Tsujimura T, Tao L, Kamikonya N, Fujiwara Y. The oncoprotein and stem cell renewal factor BMI1 associates with poor clinical outcome in oesophageal cancer patients undergoing preoperative chemoradiotherapy. BMC Cancer 2012 Oct 9;12:461-2407-12-461.
(61) Dexter SP, Sue-Ling H, McMahon MJ, Quirke P, Mapstone N, Martin IG. Circumferential resection margin involvement: an independent predictor of survival following surgery for oesophageal cancer. Gut 2001 May;48(5):667-670.
(62) National Comprehensive Cancer Network (NCCN) Guidelines Esophageal and Esophagogastric Junction Cancers. Version 2.2013 ESOPH-E 6; Available at: http://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf. Accessed 03/05, 2014.
(63) Blom RL, Sosef MN, Nap M, Lammering G, van den Berkmortel F, Hulshof MC, et al. Comparison of two neoadjuvant chemoradiotherapy regimens in patients with potentially curable esophageal carcinoma. Dis Esophagus 2013 Sep 5.
(64) Huang SD, Yuan Y, Liu XH, Gong DJ, Bai CG, Wang F, et al. Self-renewal and chemotherapy resistance of p75NTR positive cells in esophageal squamous cell carcinomas. BMC Cancer 2009 Jan 10;9:9-2407-9-9.
(65) Tang KH, Dai YD, Tong M, Chan YP, Kwan PS, Fu L, et al. A CD90(+) tumor-initiating cell population with an aggressive signature and metastatic capacity in esophageal cancer. Cancer Res 2013 Apr 1;73(7):2322-2332.
(66) Tian F, Li D, Chen J, Liu W, Cai L, Li J, et al. Aberrant expression of GATA binding protein 6 correlates with poor prognosis and promotes metastasis in cholangiocarcinoma. Eur J Cancer 2013 May;49(7):1771-1780.
(67) Zhong Y, Wang Z, Fu B, Pan F, Yachida S, Dhara M, et al. GATA6 activates Wnt signaling in pancreatic cancer by negatively regulating the Wnt antagonist Dickkopf-1. PLoS One 2011;6(7):e22129.
(68) Kimchi ET, Posner MC, Park JO, Darga TE, Kocherginsky M, Karrison T, et al. Progression of Barrett's metaplasia to adenocarcinoma is associated with the suppression of the transcriptional programs of epidermal differentiation. Cancer Res 2005 Apr 15;65(8):3146-3154.
(69) Lin L, Bass AJ, Lockwood WW, Wang Z, Silvers AL, Thomas DG, et al. Activation of GATA binding protein 6 (GATA6) sustains oncogenic lineage-survival in esophageal adenocarcinoma. Proc Natl Acad Sci U S A 2012 Mar 13;109(11):4251-4256.
(70) Vermeulen L, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol 2010 May;12(5):468-476.
(71) Herreros-Villanueva M, Zhang JS, Koenig A, Abel EV, Smyrk TC, Bamlet WR, et al. SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells.

Oncogenesis 2013 Aug 5;2:e61.
(72) Yan KS, Chia LA, Li X, Ootani A, Su J, Lee JY, et al. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci U S A 2012 Jan 10;109(2):466-471.
(73) Sims-Mourtada J, Izzo JG, Apisarnthanarax S, Wu TT, Malhotra U, Luthra R, et al. Hedgehog: an attribute to tumor regrowth after chemoradiotherapy and a target to improve radiation response. Clin Cancer Res 2006 Nov 1;12(21):6565-6572.