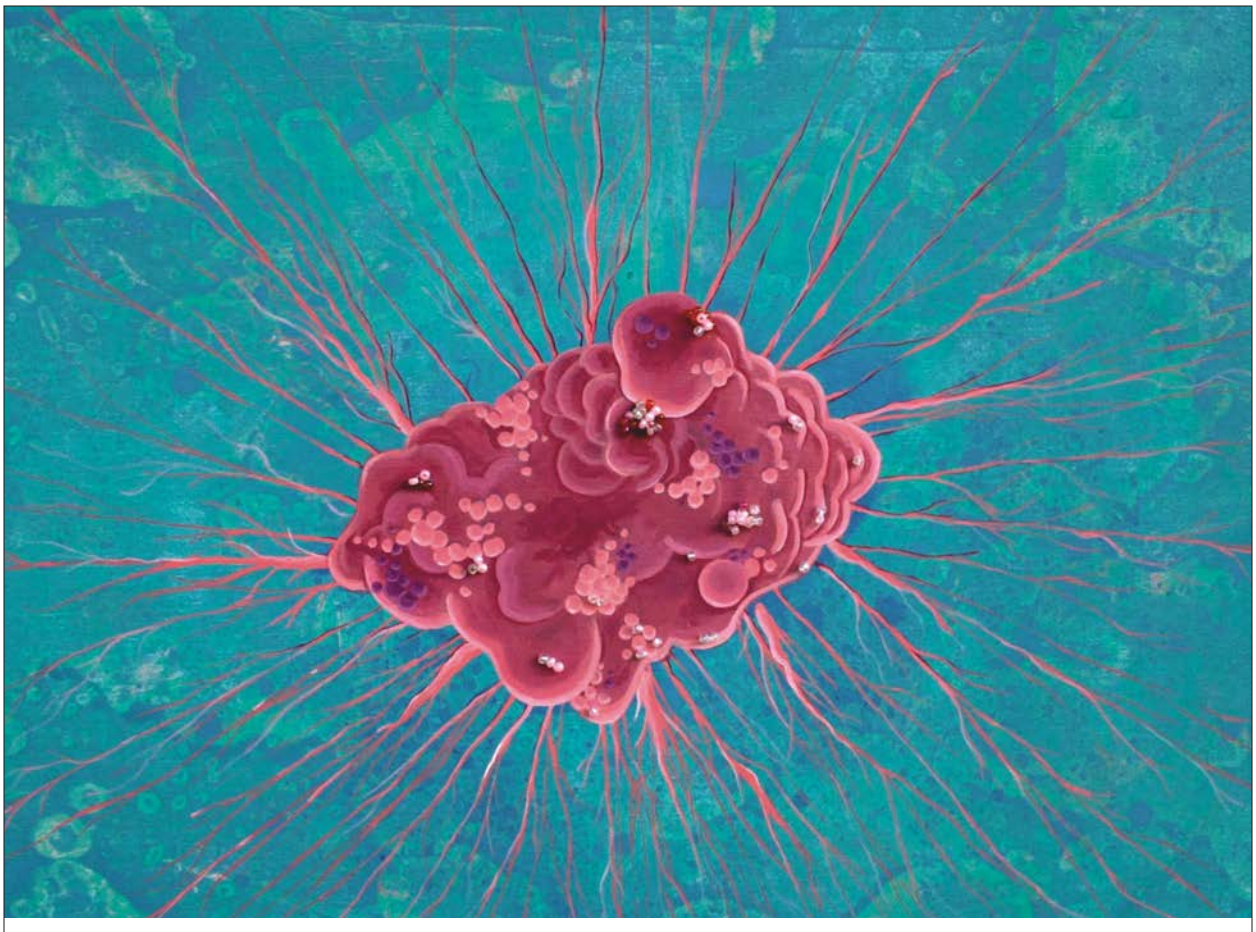

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Cancer Stem Cell: The Mastermind of Carcinogenesis

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ABSTRACT

Cancer is a malignant disease with high mortality rate and without sustainable treatment. Aberrant cell division initiates cancer that also gains the augmentation ability throughout the body by some complex biochemical and signaling processes. To elucidate cancer progression, Cancer Stem Cell (CSC) model gives comprehensive proposal about the mastermind behind cancer development and progression. Cancer stem cell is a progressive concept which unravels the transition mystery of normal cell into malignant cell. Cancer stem cell develops from epithelial cell layer by Epithelial Mesenchymal Transition (EMT) process and it invades normal cell by Msenchymal Epithelial Transition (MET). Different embryogenic signaling pathways also involves in progression of cancer stem cells. The CSC is thought to be responsible for failure of traditional cancer therapies, targeting CSC to develop more fecund treatments. In recent years, cancer researchers opting for CSC model to design new treatment in order to eradicate cancer. Targeting molecular and cellular marker of CSC or its pathway may enclose a new jurisdiction in cancer biology.

Key words: Cancer, cancer stem cell, epithelium-mesenchymal transition, mesenchymal-epithelium transition, cancer stem cell marker, bone morphogenetic proteins

INTRODUCTION

Carcinoma is one of the major cancers, originates from epithelial layer and spreads different parts of the body. In 2013, 25% deaths has been reported thus makes this diseases a devastating one (Siegel *et al*., 2013). Recurrence after treatment is main difficulty to deal with carcinoma. Accomplished biological process of recurrence and spreading of cancer cells are still mysterious. One of the mainstream hypotheses in disease progression and therapy resistance of cancer is Cancer Stem Cell (CSC).

Cancer stem cell share similar molecular properties to natural stem cells. Which denotes the recurrence and spreading features of tumor is easily unterstood. For that reason cancer initiating cells or recurrence cells termed as Cancer Stem Cells (CSCs).

So, how the cancer initiating cells originate? CSC model denoted that Cancer Stem Cell originate by the process of Epithelial Mesenchymal Transition (EMT). Emerging evidence revealed that Epithelium Mesenchymal Transition (EMT) is the precursor of cancer progression or recurrence. The EMT process in carcinoma (cancer in epithelium call) gives birth of motile, invasive and malignant mesenchymal cells. Those mesenchymal cells can invade and infect any organ of the body by circulatory blood stream, causing new tumor by reverse process MET and metastasis (Fig. 1). Different factors induced EMT process on carcinoma not only increases cancer but also

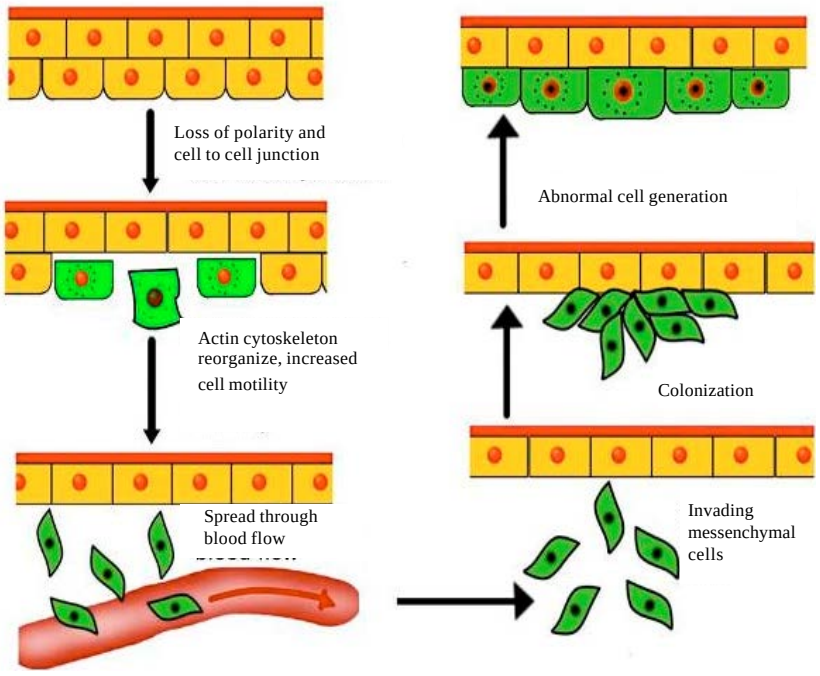

Fig. 1: Epithelial mesenchymal transition and mesenchymal epithelial transition to spread tumour growth. EMT is the precursor of originating CSCs. At first stage of EMT cell loss its polarity and cell-cell junction. In process of conversion epithelial cell gain motility, reorganize its actin cytoskeleton and transform to mesenchymal cell. On the other hand MET process involves to progression of tumour

involves in drug resistance of these cells. Mesenchymal cell in this process assume to resemble cancer stem cell (Thiery, 2003). In the current review, it was tried to establish authenticity of cancer stem cells as a major factor of cancer progression and recurrence even after therapies (Chemo). Meanwhile, this study also illustrates current status of cancer stem cell targeted therapeutics.

TUMOR PROGRESSION MODEL

Carcinoma or malignant tumor is a very common type of cancer including breast, lung, colon, prostate and ovarian cancer. Malignant tumors are characterized by histology and expression of specific markers clinically (Visvader and Lindeman, 2008). Cellular origin of most carcinoma is still unrevealed but speculation point out that different subtype origin can cause malignant tumor progression throughout the body simutaneously. Nevertheless, tumor cells show functional heterogeneity which exhibit distinctive proliferation and distinguished characteristic (Heppner and Miller, 1983). In addition of malignant tumor cells heterogeneity and regenerative capacities two models put forward: Cancer Stem Cell (CSC) and Clonal Evolution Models.

CANCER STEM CELL MODEL

Cancer stem cells model open a new chapter of cancer research. The CSCs refer to a properties of self-renewal and diverse cell generator which comprise tumor (Bonnet and Dick, 1997;

Reya *et al.*, 2001) (Fig. 2). Cancer stem cell hypothesis is an dazzling idea to understand malignant tumor functional heterogeneity. This model indicates a hierarchical organization of cell in tumor, in which stem cells subpopulation can conduct tumor growth (Visvader and Lindeman, 2008).

Origin of CSCs is different from normal cell. It refers that the origin could be the cell which receives first oncogenic hits (Visvader and Lindeman, 2008). Notably, transformation of normal stem cell doesn't initiate CSC. So, the deduction of originating CSC, heading towards EMT process in the carcinoma area. Evidence for CSC came first from acute myeloid leukemia (Lapidot *et al.*, 1994). There are distinct features of cancer stem cell in contrast of stem cells (Table 1).

Table 1: Comparisons between stem cell and cancer stem cell (Spillane and Henderson, 2007)

Feature	Stem cell	Cancer stem cell
Self-renew	Yes	Yes
Differentiate regulation	Highly	Poorly
Production	Mature tissue	Tumour
Life span	Long	Long
Apoptosis resistance	Yes	Yes

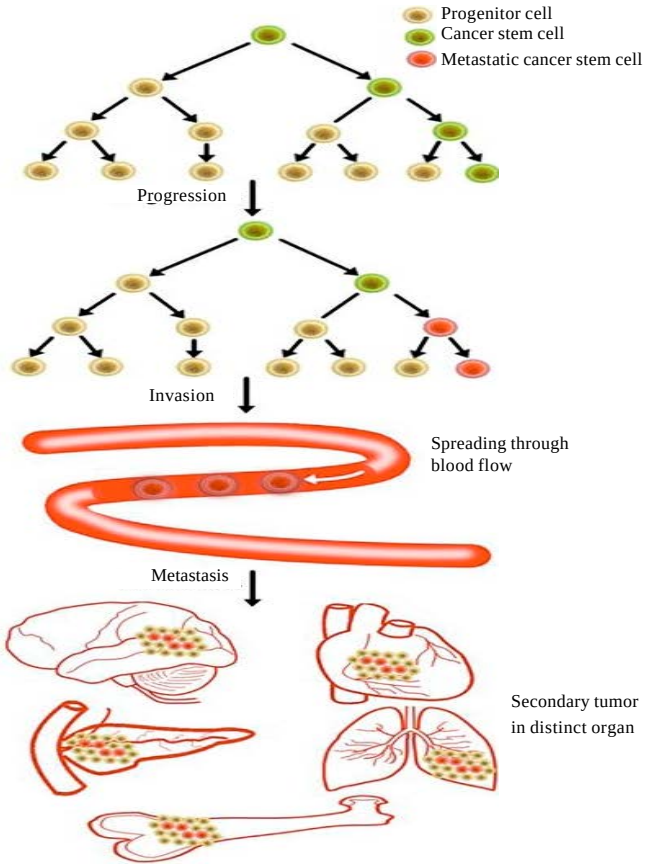

Fig. 2: Cancer stem cells differentiation and invasion. Along with tumor progression, genetic and epigenetic changes may cause the appearance of self renewing metastatic cancer stem cell, that spreads through blood flow and infest on a new organ and form a secondary tumor

CSCs model postulates that transition in between epithelial and mesenchymal generates CSCs which spread carcinoma throughout the body (Polyak and Weinberg, 2009). The distinguishing feature between CSCs model and clonal evolution model is only that CSC model is hierarchical.

EVIDENCE OF CSCs EXISTENCE

Identification of cancer stem cell is a complex work. Several processes had been applied to pick out these cells. One of the processes is the serial transplantation of invaded population into an animal model such as mouse. Speculation that CSC containing population will reestablish self renewal capability, show heterogeneity and invade other cells. Identify the existence of CSCs from this method is quite confounding. In xenotransplantation process, immunosuppressant incompleteness, species differences in cytokines and growth factor don't usually like the same. Notably syngeneic models, transplantation doesn't seem to prove an effective one because of not precisely recapitulation (Visvader and Lindeman, 2008).

Using cell marker to identify CSC is a comprehensive method than others. Number of cell markers including CD133 (also known as PROM1), CD44, CD24, E_pCAM (Epithelial cell adhesion molecule), THY1, ATP-binding cassette B5(ABCB5) proved effective to identify CSCs. Common cell marker CD133 and CD44 used to fractionate CSC in different carcinoma (Visvader and Lindeman, 2008).

CSC was first isolated and identified from breast cancer sample (Al-Hajj *et al*., 2003). Consequently, CD133 marker applied to mark in different brain tumour such as glioblastoma multiforme, pediatric medulloblastoma and ependymomas (Singh *et al*., 2004; Bao *et al*., 2006a, b; Beier *et al*., 2007; Taylor *et al*., 2005). CD133 was implemented to identify major types of cancer such as pancreatic (Hermann *et al*., 2007) carcinoma, colorectal carcinoma (O'Brien *et al*., 2006; Ricci-Vitiani *et al*., 2007).

EMBRYOGENIC SIGNALING PATHWAYS

Hedgehog signaling pathway: During embryonic development Hedgehog (Hh) signaling pathway (Fig. 3) controls tissue polarity, patterning maintenance and stem cell maintenance (Ingham and McMahon, 2001). Excessive activation of this pathway may lead to tumorigenesis, by either mutation or deregulation, has recently established. The Hh pathway involvement has also been demonstrated in different types of non mutation driven, paracrine deregulation of signaling (Yauch *et al*., 2008). The most interesting feature about Hh signaling is its involvement with cancer stem cells. Acetylation of Hh n-terminus by rasp enzyme is the first step of signal activation (Micchelli *et al*., 2002). The signaling starts by binding of Hh to the transmembrane receptor Ptch1.

Notch signaling pathway: Notch signaling has crucial significance in regulating cell to cell communication during embryogenesis and also cellular proliferation, differentiation and apoptosis (Fig. 4) (Artavanis-Tsakonas *et al*., 1999). This signaling process is also mediator for normal hematopoiesis, neural stem cell survival, immune regulation, colorectal epithelial maturation and breast development (Dontu *et al*., 2004; Androutsellis-Theotokis *et al*., 2006). The mammalian membrane bound notch ligands construct of two structurally distinct families: One is Deltalike ligands (Dlls) 1, 3 and 4 and another one is Jagged ligands 1 and 2 which adjunct with four transmembranes of notch receptors (Takebe *et al*., 2010). The coupling of notch ligand-receptors coordinates communication between adjacent cells. The extra cellular notch receptor region consists of different epidermal growth factors like domains which mediate interactions with notch ligands.

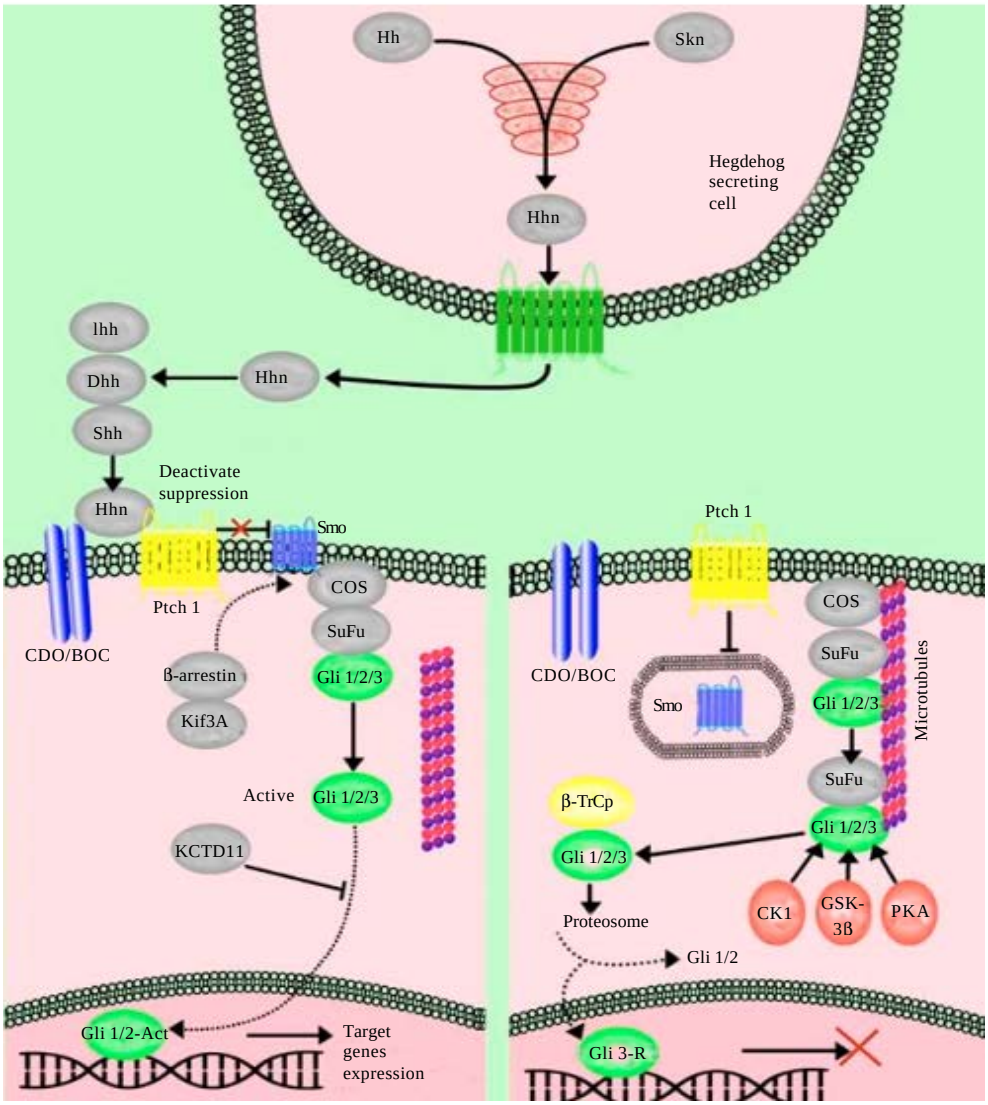

Fig. 3: Hedgehog signaling pathway exists in an active and inactive state. Activation is caused by diffusion of Hh ligands Sonic (Shh), Desert (Dhh) and Indian (Ihh). In the inactive state, patched (Ptch1) is bound to and represses smoothened (Smo). Activation of Hh target genes repressed by Smoothened (Smo) with glioma-associated (Gli) 3-repressor (Gli 3-R). In the active state, the N-terminal domain of the Hh ligand (HhN) attaches to Ptch1 and depression of Smo retards and Smo translocates to membrane, where Gli zinc finger transcription factor inhibitory kinases are unrepressed allowing translocation to nucleus and transcription of Gli target genes (Cyclin D, Cyclin E, Myc, Gli 1, Patched, HIP, VEGF, IGF1 and others)

Notch ligand and receptor affinity depends on the amount of epidermal growth factor domain fucosylation by the Fringe proteins, that is, lunatic, radical, or manic (Rampal *et al.*, 2005). Notch receptors found as hetero dimers which consists of noncovalently bound extracellular and transmembrane domains (Takebe *et al.*, 2010).

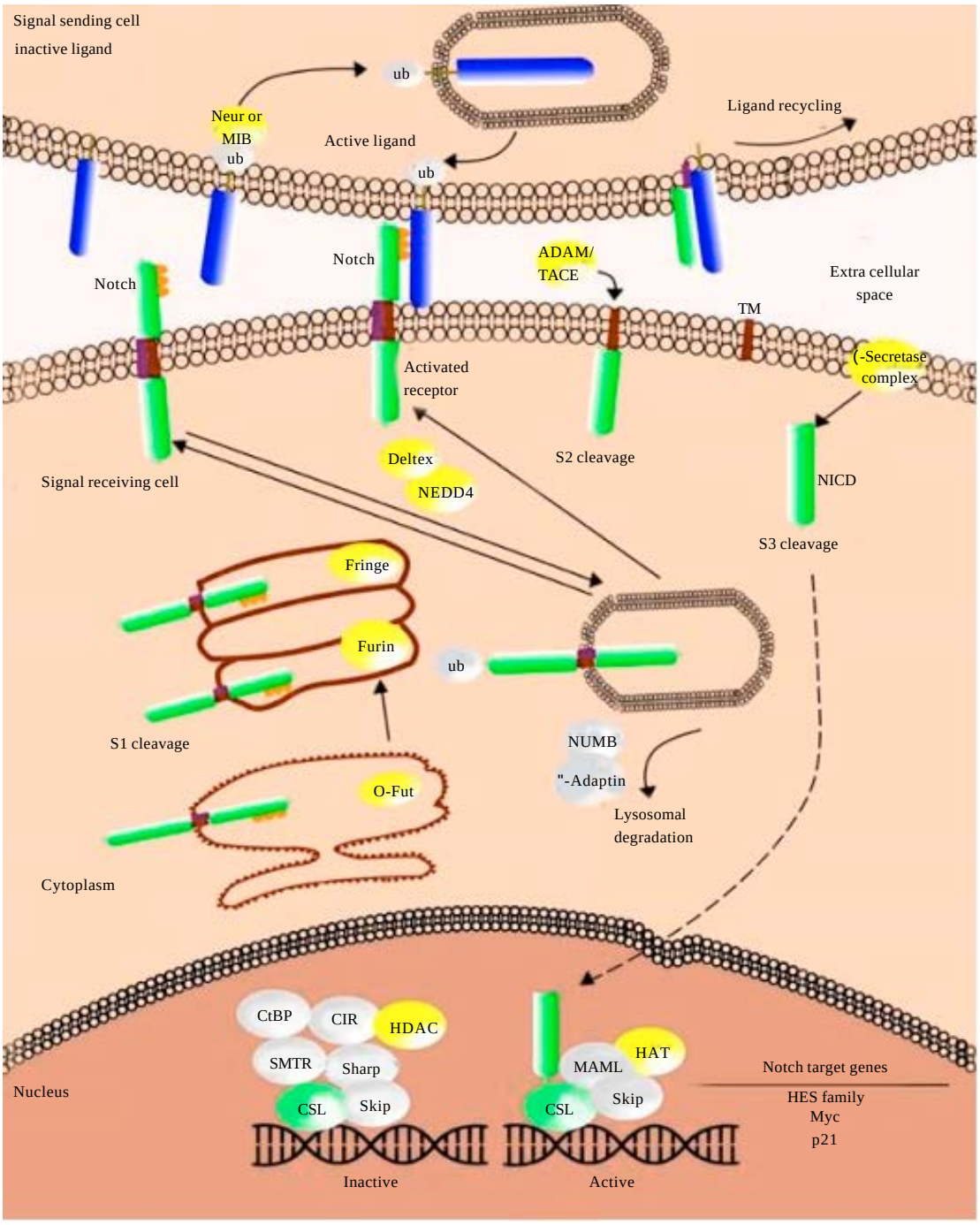

Fig. 4: Notch signaling. Activation of the Notch receptor happens by attachment of membrane-bound Delta or Jagged ligands during cell-to-cell contact. Then absorption and proteolysis of the heterodimer Notch receptor (by ADAM and γ-secretase complex) happens. Then NICD-a soluble fragment is released into the cytoplasm. The NICD moves to the nucleus where it serves as a transcriptional activator of Notch-associated target genes which includes HES, Myc and p21

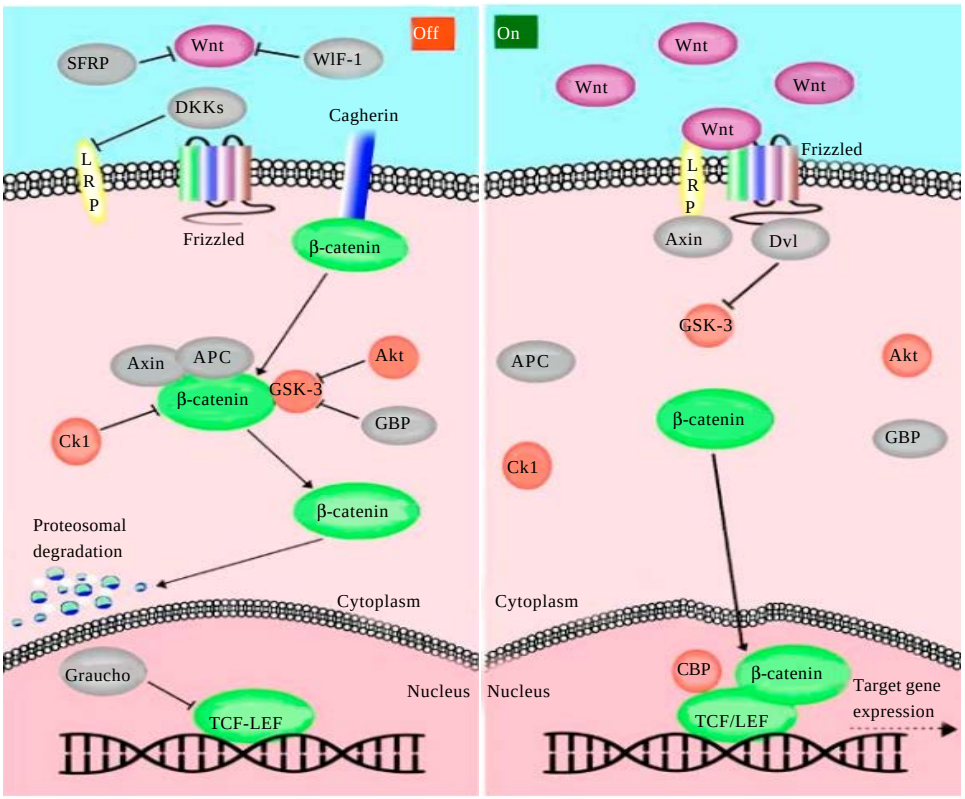

Fig. 5: Wnt signaling pathway.The pathway exists in an active state and an inactive state Wnt ligand-binding is inhibited by secretion of Frizzled-related proteins sFRPs and Wnt inhibitory factor/(WIF1). Dickkopfs (DKKs) inhibit the LDL receptor protein (LRP) directly. Secreted Wnt ligands bind to the Frizzled/LRP-5, glycogen synthase kinase 3b (GSK3b) recruitment complex which is phosphorylated and inactivates the destruction complex. This allows stability of beta-catenin followed by nuclear translocation and binding to the DNA binding transcription complex. The transcription complex contains T-cell factor/lymphoid enhancer factor (Tcf/Lef), cAMP-responsive element binding protein(CREB), B-cell lymphoma 9 (BCL9) and pygopus (Pygo). The negative regulators for the transcription complex are Groucho/histone deacetylase (HDAC)

Wnt signaling pathway: Wnt proteins consists 19 highly conserved glycoproteins, those play part as ligands for the Frizzled (Fz) transmembrane receptor (Angers and Moon, 2009). Wnt proteins moderate direct cell fate determination at different steps of development and regulate the development in a variety of organ systems including cardiovascular, Central Nervous System (CNS), renal and lung during embryogenesis (Grigoryan *et al*., 2008). Wnt signaling holds central role in the regulation of tissue selfrenewal, espicially in intestinal crypts, hair follicles and bone growth plates in adults (Clevers, 2006; Andrade *et al*., 2007). The importance of the wnt signaling pathway in multiple organ systems have demonstrated by studying loss-of-function and gain-of-function in mice with beta-catenine mutation (Grigoryan *et al*., 2008). By binding to the Fz receptors, wnt initiates two distinct signaling cascades, termed as canonical or non canonical (Fig. 5).

IDENTIFICATION OF CSC IN DIFFERENT TYPES OF CANCER

Cancer stem cells have been identified in different tissues. Stem cell marker expression has been counted to identify the organs persisted with cancer stem cells.

Identification of CSC in pancreatic carcinoma: Pancreatic carcinoma is accused to be the fourth leading cancer related mortality among various types of cancer (Hermann *et al*., 2007). Emerging evidence suggests that cancer stem cells have been implicated to spread pancreatic carcinoma in invasive mood. To identify whether the concept of CSC accurate, several experiment conducted. Stem cell marker expressions were actually observed to know the truth about them. An experiment showed that pancreatic carcinoma cell expressing CD133 which is one of the cancer stem cell marker (Hermann *et al*., 2007). Pancreatic cancer cells were collected from patient and observed their cell surface marker. The entire sample shows the presence of CD133 maker expression which is the confirmation of the presence of cancer stem cell.

Another experiment conducted to prove the evidence of CSC by xenograft method. In this process cancer cells transferred to immunocompromised mice and observed their expression, self renewal possibility. This experiment showed the positive signal about CSC. In this process cancer cells represents CD44+CD24+ESA+marker and also invasive malignant properties (Li *et al*., 2007).

Identification of CSC in breast cancer: Breast cancer is considered to be the first one from which putative cancer stem cells was isolated (Al-Hajj and Clarke, 2004). In order to identify CSC in breast carcinoma Al-hajj and his colleagues conducted an in-vitro experiment where a cell population isolated with high CD44 expression and low CD24 ($CD44^{+}CD24^{-/low}$) expression. Cell population of these was invasive, self-renewal actually presenting stem cell properties when injected into a immunocompromised mice (Al-Hajj and Clarke, 2004). This experiments carries clear evidence of cancer stem cell existence in breast carcinoma and $CD44^{+}CD24^{-/low}$ possessing stem cell like properties (Al-Hajj and Clarke, 2004).

Identification of CSC in brain tumour: Brain cancer is one of the malignant carcinoma which has high mortality among children (Singh *et al*., 2003). Singh *et al*. (2003) conducted an experiment to identify CSCs existence in brain tumour. From this experiment they got CD133+expression from the sample of brain cancer cells. Those cells shows CD133+expression are termed BSTC (Caceres-Cortes *et al*., 2001).

The cell surface markers in different cancer stem cell(s) (Isolated from different part of the human body) are summarized in Table 2 and their overall functions are listed in Table 3.

ORIGIN AND INVADING PROCESS OF CANCER STEM CELL

Origin of cancer stem cell is a matter of debate amongst most scientists. Recent experiment put some strong indication that EMT is the sole source of generating cancer stem cell. To ensure this, Mani *et al*. (2008) conducted an experiment about the outcome from EMT in non tumorigenic cell, immortalized human mammary epithelial cell. They induced Twist or Snail transcription factor. Both of these factors are capable to initiate EMT in cell (Batlle *et al*., 2000; Cano *et al*., 2000; Yang *et al*., 2004, 2006). As expected from this research, researcher found fibroblast like mesenchymal cell which also can be cell stem cell. So, if EMT occurs at cancer cells it will definitely produce Cancer Stem Cell.

Table 2: Identification of cell marker in different types of cancer stem cell (+sign indicates the corresponding cell marker find out on the corresponding carcinoma and-sign indicates cell marker didn't find out) (Al-Hajj *et al*., 2003; Ginestier *et al*., 2007; Singh *et al*., 2004; Dalerba *et al*., 2007; Ricci-Vitiani *et al*., 2007; Dean *et al*., 2005; Huang *et al*., 2009; Prince *et al*., 2007; Ho *et al*., 2007; Eramo *et al*., 2007; Ma *et al*., 2007; Yang *et al*., 2008; Li *et al*., 2007; Hermann *et al*., 2007; Gibbs *et al*., 2005; Bonnet and Dick, 1997; Hope *et al*., 2004; Kong *et al*., 2008)

	Cell marker													
Cancer	$CD19^+$	$CD24^+$	$CD24^-$	$CD34^+$	$CD38^-$	$CD44^+$	$CD90^+$	$CD105^+$	$CD133^+$	Lin^-	$ALDH1^+$	$EpCAM^+$	ESA^+	$Strol^+$
Breast	-	-	+	-	-	+	-	-	-	+	+	-	-	-
Brain	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Colorectal	-	-	-	-	-	+	-	-	+	-	+	+	-	-
Head and neck squamous cell carcinoma	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Lung	-	+	-	-	-	+	-	-	+	-	-	-	-	-
Liver	-	-	-	-	-	+	+	-	+	-	-	-	-	-
Pancreatic	-	+	-	-	-	+	-	-	+	-	-	-	+	-
Sarcomas	-	-	-	-	-	+	-	+	-	-	-	-	-	+
Leukemia	+	-	-	+	+	-	-	-	-	-	-	-	-	-

Table 3: Different cancer stem cell marker with their relative fuctions

Cancer stem cell markers	Functions
CD19 (B-lymphocyte antigen CD19)	CD19 is a 556 amino acid containing protein. Its main function is to assemble with the antigen receptor of B-lymphocyte so that it can decrease the threshold for antigen receptor-dependent stimulation
CD24 (Signal transducer CD24/small cell lung carcinoma cluster 4 antigen)	CD24 regulates B-cell activation responses, promotes AG-dependent B-cell proliferation and also prevent the terminal differentiation of B-cell into antibody forming cells. It has 80 amino acid in its sequence
CD34 (Hematopoietic progenitor cell antigen CD34)	CD34 is an adhesion molecule Which plays a role in early hematopoiesis. This protein could act as a scaffold for the attachment of lineage specific glycans that allowing stem cells to bind to lectins. Also presents carbohydrate ligands to selectins
CD38 (ADP-ribosyl cyclase 1)	CD38 can synthesize cyclic ADP-ribose. Cyclic ADP-ribose is a second messenger for glucose-induced insulin secretion. This protein also has cADPr hydrolase activity
CD44 (CD44 antigen/Epican)	CD44 is a receptor for hyaluronic acid (HA). Cell-cell and cell-matrix interactions implicated through its affinity for HA. In cell migration, tumour growth and progression, adhesion with HA plays an important role.CD44 plays a viatl role in cancer by forming invadopodia.. CD44 protein aslo involved in lymphocyte activation, recirculation and homing
CD90 (Thy-1 membrane glycoprotein)	During synaptogenesis, CD90 may play a role in cell-cell or cell-ligand interaction and other events in the brain
CD105 (Endoglin)	CD105 is one of the major glycoprotein of vascular endothelium. CD105 regulate angiogenesis and may play a critical role in the binding of endothelial cells to integrins and/or other RGD receptors. This protein also acts as TGF-beta coreceptor and is involved in the TGF-beta/BMP signaling cascade
CD133 (Prominin-1)	CD133 cancer stem cell marker binds cholesterol in cholesterol-containing plasma membrane microdomains. CD133 may play a role in apical plasma membrane organization of epithelial cells. Also act as akey regulator of disk morphogenesis during early retinal development. Regulate of MAPK and Akt signaling pathways and in neuroblastoma cells suppresses cell differentiation
ALDH1	ALDH1 catalyse the oxidation of aliphatic and aromatic aldehydes to carboxylic acids. ALDH 1 plays an important role to convert retinol to retinoic acid. Retinoic acid is important for proliferation, differentiation and survival

Table 3: Continue

Cancer stem cell markers	Functions
EpCAM (Epithelial cell molecule)	Act as hemophilic interaction molecule between intestinal epithelial cells (IECs) and adhesion intraepithelial lymphocytes (IELs) at the mucosal epithelium which provides immunological barrier against mucosal infection. This protein also plays a role in embryonic stem cells proliferation and differentiation
ESA (Flotillin-2)	ESA may act as Scaffolding protein within caveolar membranes. ESA may be involved in epidermal cell adhesion and epidermal structure and function
ABCG5	ABCG5 is a member of ATP binding cassette family which transports sterol and other lipids. ABCG5 also known as breast cancer resistance protein

Epithelial cells are closely adjacent to each other to form like a cobblestone wall, creating less intercellular space. In comparison mesenchymal cells have the absence of cell junction, having cell motility power. In this process epithelial cell firstly, lose its polarity and cell-cell junction after that actin cytoskeleton reorganized and increased cell motility turned epithelium cells into mesenchymal (Thiery, 2003).

Mesenchymal cells or Cancer stem Cells spreads throughout the body by blood steam and conducting reverse process called mesenchymal-epithelium transition (MET). Mesenchymal epithelium transition (MET) is implicated to structure heart, somites and kidney ontogenesis. By which tumour develops rapidly throughout the body (Thiery, 2003).

TARGETING CSC FOR THERAPEUTICS

Cancer stem cells posses stem cell properties and found chemotherapy and radiotherapy resistant (Moltzahn *et al.*, 2008). Not only that the patient who receive chemotherapy often relapse. Several drug also resulted in an enrichment of CSCs in tumour (Dexter and Leith, 1986). For example oxaliplatin treatment of colon cancer increased the abundance of CSCs by more than 10 times (Dean *et al.*, 2005). Quiescent CSCs are more chemo and radio resistant in comparison with CSCs (Visvader and Lindeman, 2008). Recent studies found that certain CSCs may undergo quiescent state and remain therapy resistant in order to initiate further damage (Ishikawa *et al.*, 2007). Conventional therapeutics isn't sufficient to treat against cancer. Proper studies of CSCs pathway, regulatory gene and surface protein may enclose new therapeutics or drugs to treat cancer.

Targeting CSCs surface protein: Cancer stem cells surface or associated protein has been extensively used for identifying or monitoring the presence of CSC (Visvader and Lindeman, 2008). CD24, CD44, CD133, ALDH1 EpCAM are important cellular markers express in cancer stem cells (Hu and Fu, 2012). Cell surface protein expression of theses marker is not a stable trait. Perhaps it may vary in different stages of cancer (O'Connor *et al.*, 2014). Notably, these surface proteins is heterogenous not only vary among different patients with same type of cancer but also within the same tumour mass (Visvader and Lindeman, 2012). Targeting cancer stem cell is therefore a tough task because of the cellular marker provides very few feasible options. Other therapeutic targeting options are targeting quiescence, self-renewal pathways and differentiation therapy (O'Connor *et al.*, 2014).

Although, targeting CSC is not an easy task, some research showed that CSC can be selectively targeted without harming the normal stem cell. Jordan and his colleagues identified a naturally occurring small molecule, named parthenolide which can selectively targets leukaemia stem cell and

not the normal stem cell (Guzman *et al*., 2005). Another molecule named rapamycin targets mTOR which led to the eradication of leukaemia initiating cells (Yilmaz *et al*., 2006). Notably, CD44 is a suitable cellular marker to target for cancer stem cell in solid cancer (Jin *et al*., 2006; Krause *et al*., 2006).

Targeting CSC signaling pathway: Because tumor cells are known to appropriate normal cellular mechanisms for growth, survival and proliferation, no large leap of faith is required to hypothesize that embryonic signaling pathways are critical to these same processes for maintenance, proliferation and quiescence in the Cancer Stem Cell (CSC). Emerging evidence supports the role of the Hedgehog, Notch and Wnt signaling pathways which are genetically conserved (Reya *et al*., 2001).

- **Hedgehog pathway:** The Hh signaling pathway is a crucial mediator of normal tissue development (Ingham and McMahon, 2001). Hh signaling may provide a unique mechanism-based therapy, blocking tumor growth and stimulating tumor regression without toxic effects on normal adjacent tissue. The prototype of Hh pathway-specific inhibitors is cyclopamine, a plant-derived steroidal alkaloid which directly binds to and inactivates Smo (Taipale *et al*., 2000). Other pathway inhibitors are under investigation as well (Rubin and de Sauvage, 2006; Kiselyov, 2006). It is important to note that results from *in vitro* studies should be interpreted with caution because in some experiments, higher concentrations of Hh antagonists were needed to inhibit cell proliferation (Evangelista *et al*., 2006; Chari and McDonnell, 2007; Rubin and de Sauvage, 2006; Lauth and Toftgard, 2007) than Hh activation, indicating the potential nonspecific or toxic effects of these compounds
- **Notch pathway:** The role of Notch signaling in cancer and current efforts to target Notch therapeutically has been reviewed recently (Rizzo *et al*., 2008). Drugs that inhibit Notch signaling are in early clinical development and others are in the pipeline. In glioblastomas, Notch expression has been associated with high nestin levels and is linked to a poor prognosis (Phillips *et al*., 2006; Shih and Holland, 2006; Romer *et al*., 2004; Sasai *et al*., 2006). Notch inhibition reduced the tumorigenicity of brain CSCs. In breast cancer, Notch expression and activation has been associated with a poor prognosis and Notch inhibitors can kill breast cancer cells *in vitro* and *in vivo* (Rizzo *et al*., 2008; Dickson *et al*., 2007; Reedijk *et al*., 2005). Farnie and co workers reported that activated Notch-1, Notch-4 and Notch target Hes-1 were expressed in mammospheres from ductal carcinoma in situ samples but not from normal breast tissue. Notch inhibition with a gamma-secretase inhibitor (GSI) or a neutralizing Notch-4 antibody reduced the ability of ductal carcinoma in situ-derived cells to form mammospheres (Farnie *et al*., 2007). These results suggest that Notch inhibition may be able to preferentially target breast CSCs

One of the reasons for the increasing interest in the therapeutic targeting of Notch in solid tumors is that, at least in some cases such as breast cancer, Notch regulates survival and proliferation in "bulk" cancer cells, as well as CSCs (Farnie *et al*., 2005, 2007; Sansone *et al*., 2007). At the same time, Notch plays a proangiogenic role in tumor endothelial cells, largely dependent on ligand Delta-4 (Noguera-Troise *et al*., 2007; Ridgway *et al*., 2006). Thus, pharmacologic inhibition of Notch signaling may have significant therapeutic effects in primary lesions, prevent the self-renewal of CSCs responsible for recurrence and metastatic disease and achieve an antiangiogenic effect (Farnie *et al*., 2007; Rizzo *et al*., 2008).

- **Wnt pathway:** A variety of compounds have been evaluated for their ability to disrupt the Wnt/beta-catenin signaling pathway in tumor cells. These compounds include imatinib which was originally identified as a platelet-derived growth factor receptor inhibitor. Imatinib also has been reported to inhibit the tyrosine phosphorylation of betacatenin (Rao *et al.*, 2006). Celecoxib, a cyclooxygenase-2 inhibitor, also was shown to inhibit beta-catenin activity in human colon carcinoma cells by inducing its degradation (Maier *et al.*, 2005). Antisense inhibitors have been evaluated against colon, esophageal, leukemia and lymphoma cells *in vitro*, resulting in decreased expression of beta-catenin (Steinbach *et al.*, 2000; Green *et al.*, 2001; Phillips *et al.*, 2002). NSAIDs also have been reported to inhibit the Wnt/beta catenin signaling pathway. Both aspirin and indomethacin inhibited beta-catenin transcription (Dihlmann *et al.*, 2001). Thiazolidinedione was reported to completely inhibit metastases in a colon cancer xenograft model by inducing the translocation of betacatenin from the nucleus to the plasma membrane. The novel compound AV65 also has shown activity to induce apoptosis in a dose-dependent manner against chronic myeloid leukemia cell lines (Kimura *et al.*, 2008). AV65 was reported to be a promising agent for the treatment of patients who are resistant to both imatinib and second-generation Abl tyrosine kinase inhibitor. Clearly, developing compounds that disrupt the Wnt/beta-catenin signaling cascade hold great promise for cancer therapeutics (Yoshizumi *et al.*, 2004)

BMP inhibition: Bone Morphogenetic Proteins (BMPs) are another way to treat with cancer stem cell (Fig. 6). Bone morphogenetic proteins induce differentiation of CD133+cells to astrocyte like cells and therefore attenuating their ability of tumour forming (Piccirillo *et al.*, 2006). Transplantation of CD133+cells with BMPs showed that the growth of tumour reduced and the survival of xenograft animals increased (Visvader and Lindeman, 2008). Another research showed that deregulation of BMPs signaling in gliomas may lead to the inhibition of differentiation of cancer stem cells and conveying pro-proliferative signals (Lee *et al.*, 2008).

CONTROVERSY OVER CSC MODEL

Origin of cancer from a specific entity was first implied Stephen Paget in the 19th century. He constructed seed and soil theory but unfortunately rejected by rest of the cancer scientist (Paget, 1889). The CSC concept re-emerged at 20th century for scientific and technological advancement. In mid-1990s Bonnet and dick provided a definitive advancement on CSC model. Later Al-Hajj and his colleagues also put some strong evidence on this topic. All these studies made an assay to find out cancer stem cell in immunodeficiency mice. These assays also accepted as gold standard for studying the digress of stemness (O'Connor *et al.*, 2014).

Despite having many experimental evidence, CSC model still a matter of debate because of some unanswered question. The followings are the matter of debate on CSC model:

- According to CSC model cancer initiates from distinct rare cell(s) called cancer stem cell(s). But in some malignant tumour the cancer stimulating cells are quite numerous in number which negates the hierarchical model of CSC (Quintana *et al.*, 2008)
- The assay technique to study CSC actually made a focal point of debate. In cancer stem cell assay, cancer stem cell found only in the immunodeficient or immunocompromised organism like the immunodeficient mice but not in the original place where it originates (O'Connor *et al.*, 2014)

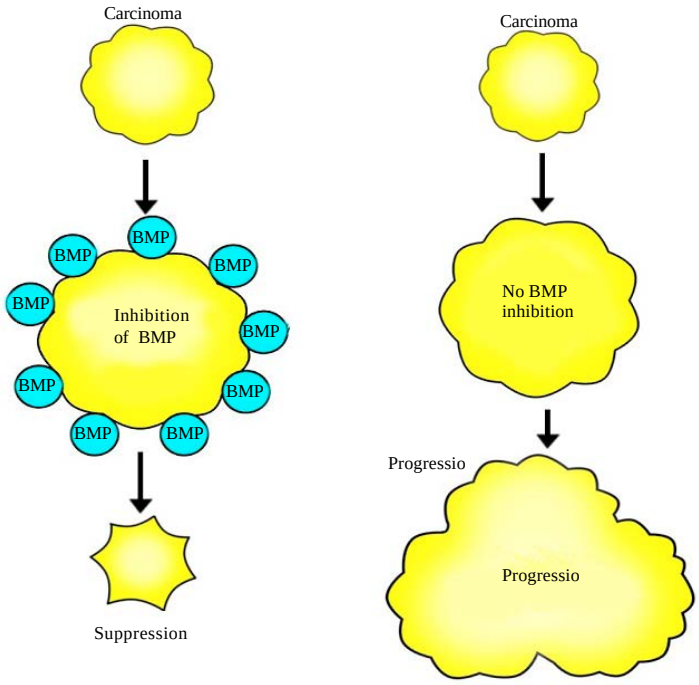

Fig. 6: Functions of BMP may cause reduced growth of carcinoma by differentiating CSC to another cell orcausing cell cycle arrest. Bone morphogenetic protein is an important factor to suppress tumour to suppress tumour growth. It induces cancer stem cell to differentiate other cell types and thus inhibit tumour growth

- Cancer Stem cell may further result under specific tools of xenotransplantation such as proteolytic enzyme, length of incubation and temperature even at same type of tumour (O'Connor *et al*., 2014)

CONCLUSION

Cancer is still remaining a disease without proper treatment. The most fatal part of cancer or malignant tumour is its recurrence ability. Despite of some strong evidences on CSC model, it's still a matter of debate. Nano base therapies by targeting CSC can be a very exciting field of research. Profound study on CSC molecular biology can lead us to discover a new sustainable treatment for cancer patients.

REFERENCES

Al-Hajj, M. and M.F. Clarke, 2004. Self-renewal and solid tumor stem cells. Oncogene, 23: 7274-7282.

Al-Hajj, M., M.S. Wicha, A. Benito-Hernandez, S.J. Morrison and M.F. Clarke, 2003. Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci., 100: 3983-3988.

Andrade, A.C., O. Nilsson, K.M. Barnes and J. Baron, 2007. Wnt gene expression in the post-natal growth plate: Regulation with chondrocyte differentiation. Bone, 40: 1361-1369.

Androutsellis-Theotokis, A., R.R. Leker, F. Soldner, D.J. Hoeppner and R. Ravin *et al*., 2006. Notch signalling regulates stem cell numbers *in vitro* and *in vivo*. Nature, 442: 823-826.

Angers, S. and R.T. Moon, 2009. Proximal events in Wnt signal transduction. Nat. Rev. Mol. Cell Biol., 10: 468-477.

Artavanis-Tsakonas, S., M.D. Rand and R.J. Lake, 1999. Notch signaling: Cell fate control and signal integration in development. Science, 284: 770-776.

Bao, S., Q. Wu, R.E. McLendon, Y. Hao and Q. Shi *et al.*, 2006a. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature, 444: 756-760.

Bao, S., Q. Wu, S. Sathornsumetee, Y. Hao and Z. Li *et al.*, 2006b. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res., 66: 7843-7848.

Batlle, E., E. Sancho, C. Franci, D. Dominguez, M. Monfar, J. Baulida and A.G. de Herreros, 2000. The transcription factor snail is a repressor of *E-cadherin* gene expression in epithelial tumour cells. Nat. Cell Biol., 2: 84-89.

Beier, D., P. Hau, M. Proescholdt, A. Lohmeier and J. Wischhusen *et al.*, 2007. $CD133^{+}$ and $CD133^{-}$ glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res., 67: 4010-4015.

Bonnet, D. and J.E. Dick, 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med., 3: 730-737.

Caceres-Cortes, J.R., J.A. Alvarado-Moreno, K. Waga, R. Rangel-Corona and A. Monroy-Garcia *et al.*, 2001. Implication of tyrosine kinase receptor and steel factor in cell density-dependent growth in cervical cancers and leukemias. Cancer Res., 61: 6281-6289.

Cano, A., M.A. Perez-Moreno, I. Rodrigo, A. Locascio and M.J. Blanco *et al.*, 2000. The transcription factor snail controls epithelial-mesenchymal transitions by repressing *E-cadherin* expression. Nat. Cell Biol., 2: 76-83.

Chari, N.S. and T.J. McDonnell, 2007. The sonic hedgehog signaling network in development and neoplasia. Adv. Anatom. Pathol., 14: 344-352.

Clevers, H., 2006. Wnt/β-catenin signaling in development and disease. Cell, 127: 469-480.

Dalerba, P., S.J. Dylla, I.K. Park, R. Liu and X. Wang *et al.*, 2007. Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. USA., 104: 10158-10163.

Dean, M., T. Fojo and S. Bates, 2005. Tumour stem cells and drug resistance. Nat. Rev. Cancer, 5: 275-284.

Dexter, D.L. and J.T. Leith, 1986. Tumor heterogeneity and drug resistance. Clin. Oncol., 4: 244-257.

Dickson, B.C., A.M. Mulligan, H. Zhang, G. Lockwood, F.P. O'Malley, S.E. Egan and M. Reedijk, 2007. High-level JAG1 mRNA and protein predict poor outcome in breast cancer. Modern Pathol., 20: 685-693.

Dihlmann, S., A. Siermann and M. von Knebel Doeberitz, 2001. The nonsteroidal anti-inflammatory drugs aspirin and indomethacin attenuate β-catenin/TCF-4 signaling. Oncogene, 20: 645-653.

Dontu, G., K.W. Jackson, E. McNicholas, M.J. Kawamura, W.M. Abdallah and M.S. Wicha, 2004. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res., 6: R605-R615.

Eramo, A., F. Lotti, G. Sette, E. Pilozzi and M. Biffoni *et al.*, 2007. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ., 15: 504-514.

Evangelista, M., H. Tian and F.J. de Sauvage, 2006. The hedgehog signaling pathway in cancer. Clin. Cancer Res., 12: 5924-5928.

Farnie, G., K. Brennan, R.B. Clarke and N.J. Bundred, 2005. Ductal Carcinoma *In situ* (DCIS) mammosphere formation effect of Epidermal Growth Factor (EGF) and Notch signaling pathways on self-renewal capacity. Breast Cancer Res. Treat., 94: S14-S14.

Farnie, G., R.B. Clarke, K. Spence, N. Pinnock, K. Brennan, N.G. Anderson and N.J. Bundred, 2007. Novel cell culture technique for primary ductal carcinoma in situ: Role of Notch and epidermal growth factor receptor signaling pathways. J. Natl. Cancer Inst., 99: 616-627.

Gibbs, C.P., V.G. Kukekov, J.D. Reith, O. Tchigrinova and O.N. Suslov *et al.*, 2005. Stem-like cells in bone sarcomas: Implications for tumorigenesis. Neoplasia, 7: 967-976.

Ginestier, C., M.H. Hur, E. Charafe-Jauffret, F. Monville and J. Dutcher *et al.*, 2007. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell, 1: 555-567.

Green, D.W., H. Roh, J.A. Pippin and J.A. Drebin, 2001. β-catenin antisense treatment decreases β-catenin expression and tumor growth rate in colon carcinoma xenografts. J. Surg. Res., 101: 16-20.

Grigoryan, T., P. Wend, A. Klaus and W. Birchmeier, 2008. Deciphering the function of canonical Wnt signals in development and disease: Conditional loss-and gain-of-function mutations of β-catenin in mice. Genes Dev., 22: 2308-2341.

Guzman, M.L., R.M. Rossi, L. Karnischky, X. Li, D.R. Peterson, D.S. Howard and C.T. Jordan, 2005. The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood, 105: 4163-4169.

Heppner, G.H. and B.E. Miller, 1983. Tumor heterogeneity: Biological implications and therapeutic consequences. Cancer Metastasis Rev., 2: 5-23.

Hermann, P.C., S.L. Huber, T. Herrler, A. Aicher and J.W. Ellwart *et al.*, 2007. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell, 1: 313-323.

Ho, M.M., A.V. Ng, S. Lam and J.Y. Hung, 2007. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res., 67: 4827-4833.

Hope, K.J., L. Jin and J.E. Dick, 2004. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat. Immunol., 5: 738-743.

Hu, Y. and L. Fu, 2012. Targeting cancer stem cells: A new therapy to cure cancer Patients. Am. J. Cancer Res., 2: 340-356.

Huang, E.H., M.J. Hynes, T. Zhang, C. Ginestier and G. Dontu *et al.*, 2009. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic Stem Cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res., 69: 3382-3389.

Ingham, P.W. and A.P. McMahon, 2001. Hedgehog signaling in animal development: Paradigms and principles. Genes Dev., 15: 3059-3087.

Ishikawa, F., S. Yoshida, Y. Saito, A. Hijikata and H. Kitamura *et al.*, 2007. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat. Biotechnol., 25: 1315-1321.

Jin, L., K.J. Hope, Q. Zhai, F. Smadja-Joffe and J.E. Dick, 2006. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat. Med., 12: 1167-1174.

Kimura, S., R. Nagao, M. Takeuchi, R. Tanaka and A. Yokota *et al.*, 2008. A novel β-catenin inhibitor, AV65 suppresses the growth of CML cell lines which acquire Imatinib-resistance because of Abl Kinase domain mutations including T315I and hypoxia-adaptation. Blood, Vol. 112.

Kiselyov, A.S., 2006. Targeting the hedgehog signaling pathway with small molecules. Anticancer Agents Med. Chem., 6: 445-449.

Kong, Y., S. Yoshida, Y. Saito, Y. Nagatoshi and M. Fukata *et al.*, 2008. $CD34^+$ $CD38^+$ $CD19^+$ as well as $CD34^+$ $CD38^-$ $CD19^+$ cells are leukemia-initiating cells with self-renewal capacity in human B-precursor ALL. Leukemia, 22: 1207-1213.

Krause, D.S., K. Lazarides, U.H. von Andrian and R.A. van Etten, 2006. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. Nat. Med., 12: 1175-1180.

Lapidot, T., C. Sirard, J. Vormoor, B. Murdoch and T. Hoang *et al.*, 1994. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature, 367: 645-648.

Lauth, M. and R. Toftgard, 2007. The Hedgehog pathway as a drug target in cancer therapy. Curr. Opin. Invest. Drugs, 8: 457-461.

Lee, J., M.J. Son, K. Woolard, N.M. Donin and A. Li *et al.*, 2008. Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell, 13: 69-80.

Li, C., D.G. Heidt, P. Dalerba, C.F. Burant and L. Zhang *et al.*, 2007. Identification of pancreatic cancer stem cells. Cancer Res., 67: 1030-1037.

Ma, S., K.W. Chan, L. Hu, T.K.W. Lee and J.Y.H. Wo *et al.*, 2007. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology, 132: 2542-2556.

Maier, T.J., A. Janssen, R. Schmidt, G. Geisslinger and S. Grosch, 2005. Targeting the beta-catenin/APC pathway: a novel mechanism to explain the cyclooxygenase-2-independent anticarcinogenic effects of celecoxib in human colon carcinoma cells. FASEB J., 19: 1353-1355.

Mani, S.A., W. Guo, M.J. Liao, E.N. Eaton and A. Ayyanan *et al.*, 2008. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell, 133: 704-715.

Micchelli, C.A., I. The, E. Selva, V. Mogila and N. Perrimon, 2002. *rasp*, A putative transmembrane acyltransferase, is required for Hedgehog signaling. Development, 129: 843-851.

Moltzahn, F.R., J.P. Volkmer, D. Rottke and R. Ackermann, 2008. Cancer stem cells: Lessons from Herculesto fight the Hydra. Urol. Oncol., 26: 581-589.

Noguera-Troise, I., C. Daly, N.J. Papadopoulos, S. Coetzee and P. Boland *et al.*, 2007. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Novartis Found. Symp., 283: 106-120.

O'Brien, C.A., A. Pollett, S. Gallinger and J.E. Dick, 2006. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature, 445: 106-110.

O'Connor, M.L., D. Xiang, S. Shigdar, J. Macdonald and Y. Li *et al.*, 2014. Cancer stem cells: A contentious hypothesis now moving forward. Cancer Lett., 344: 180-187.

Paget, S., 1889. The distribution of secondary growths in cancer of the breast. Lancet, 133: 571-573.

Phillips, H.S., S. Kharbanda, R. Chen, W.F. Forrest and R.H. Soriano *et al.*, 2006. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression and resemble stages in neurogenesis. Cancer Cell, 9: 157-173.

Phillips, R.K.S., M.H. Wallace, P.M. Lynch, E. Hawk and G.B. Gordon *et al.*, 2002. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. Gut, 50: 857-860.

Piccirillo, S.G.M., B.A. Reynolds, N. Zanetti, G. Lamorte and E. Binda *et al.*, 2006. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature, 444: 761-765.

Polyak, K. and R.A. Weinberg, 2009. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat. Rev. Cancer, 9: 265-273.

Prince, M.E., R. Sivanandan, A. Kaczorowski, G.T. Wolf, M.J. Kaplan *et al.*, 2007. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc. Natl. Acad. Sci. USA., 104: 973-978.

Quintana, E., M. Shacketon, M.S. Sabel, D.R. Fullen, T.M. Johnson and S.J. Morrison, 2008. Efficient tumour formation by single human melanoma cells. Nature, 456: 593-598.

Rampal, R., J.F. Arboleda-Velasquez, A. Nita-Lazar, K.S. Kosik and R.S. Haltiwanger, 2005. Highly conserved O-fucose sites have distinct effects on Notch1 function. J. Biol. Chem., 280: 32133-32140.

Rao, A.S., N. Kremenevskaja, R. von Wasielewski, V. Jakubcakova, S. Kant, J. Resch and G. Brabant, 2006. Wnt/β-catenin signaling mediates antineoplastic effects of imatinib mesylate (gleevec) in anaplastic thyroid cancer. J. Clin. Endocrinol. Metab., 91: 159-168.

Reedijk, M., S. Odorcic, L. Chang, H. Zhang and N. Miller *et al.*, 2005. High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res., 65: 8530-8537.

Reya, T., S.J. Morrison, M.F. Clarke and I.L. Weissman, 2001. Stem cells, cancer and cancer stem cells. Nature, 414: 105-111.

Ricci-Vitiani, L., D.G. Lombardi, E. Pilozzi, M. Biffoni, M. Todaro, C. Peschle and R. De Maria, 2007. Identification and expansion of human colon-cancer-initiating cells. Nature, 445: 111-115.

Ridgway, J., G. Zhang, Y. Wu, S. Stawicki and W.C. Liang *et al.*, 2006. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. Nature, 444: 1083-1087.

Rizzo, P., H. Miao, G. D'Souza, C. Osipo and J. Yun *et al.*, 2008. Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. Cancer Res., 68: 5226-5235.

Romer, J.T., H. Kimura, S. Magdaleno, K. Sasai and C. Fuller *et al.*, 2004. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in $Ptc1^{+/-}p53^{-/-}$ mice. Cancer Cell, 6: 229-240.

Rubin, L.L. and F.J. de Sauvage, 2006. Targeting the Hedgehog pathway in cancer. Nat. Rev. Drug Discovery, 5: 1026-1033.

Sansone, P., G. Storci, S. Tavolari, T. Guarnieri and C. Giovannini *et al.*, 2007. IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. J. Clin. Invest., 117: 3988-4002.

Sasai, K., J.T. Romer, Y. Lee, D. Finkelstein, C. Fuller, P.J. McKinnon and T. Curran, 2006. Shh pathway activity is down-regulated in cultured medulloblastoma cells: Implications for preclinical studies. Cancer Res., 66: 4215-4222.

Shih, A.H. and E.C. Holland, 2006. Notch signaling enhances nestin expression in gliomas. Neoplasia, 8: 1072-1082.

Siegel, R., D. Naishadham and A. Jemal, 2013. Cancer statistics, 2013. CA: Cancer J. Clin., 63: 11-30.

Singh, S.K., C. Hawkins, I.D. Clarke, J.A. Squire and J. Bayani *et al.*, 2004. Identification of human brain tumour initiating cells. Nature, 432: 396-401.

Singh, S.K., I.D. Clarke, M. Terasaki, V.E. Bonn, C. Hawkins, J. Squire and P.B. Dirks, 2003. Identification of a cancer stem cell in human brain tumors. Cancer Res., 63: 5821-5828.

Spillane, J.B. and M.A. Henderson, 2007. Cancer stem cells: A review. ANZ J. Surg., 77: 464-468.

Steinbach, G., P.M. Lynch, R.K. Phillips, M.H. Wallace and E. Hawk *et al.*, 2000. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N. Engl. J. Med., 342: 1946-1952.

Taipale, J., J.K. Chen, M.K. Cooper, B. Wang and R.K. Mann *et al.*, 2000. Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature, 406: 1005-1009.

Takebe, N., P.J. Harris, R.Q. Warren and S.P. Ivy, 2010. Targeting cancer stem cells by inhibiting Wnt, notch and hedgehog pathways. Nat. Rev. Clin. Oncol., 8: 97-106.

Taylor, M.D., H. Poppleton, C. Fuller, X. Su and Y. Liu *et al.*, 2005. Radial glia cells are candidate stem cells of ependymoma. Cancer Cell, 8: 323-335.

Thiery, J.P., 2003. Epithelial-mesenchymal transitions in development and pathologies. Curr. Opin. Cell Biol., 15: 740-746.

Visvader, J.E. and G.J. Lindeman, 2008. Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. Nat. Rev. Cancer, 8: 755-768.

Visvader, J.E. and G.J. Lindeman, 2012. Cancer stem cells: Current status and evolving complexities. Cell Stem Cell, 10: 717-728.

Yang, J., S.A. Mani, J.L. Donaher, S. Ramaswamy and R.A. Itzykson *et al.*, 2004. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell, 117: 927-939.

Yang, J., S.A. Mani and R.A. Weinberg, 2006. Exploring a new twist on tumor metastasis. Cancer Res., 66: 4549-4552.

Yang, Z.F., D.W. Ho, M.N. Ng, C.K. Lau and W.C. Yu *et al.*, 2008. Significance of $CD90^+$ cancer stem cells in human liver cancer. Cancer Cell, 13: 153-166.

Yauch, R.L., S.E. Gould, S.J. Scales, T. Tang and H. Tian *et al.*, 2008. A paracrine requirement for hedgehog signalling in cancer. Nature, 455: 406-410.

Yilmaz, O.H., R. Valdez, B.K. Theisen, W. Guo, D.O. Ferguson, H. Wu and S.J. Morrison, 2006. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature, 441: 475-482.

Yoshizumi, T., T. Ohta, I. Ninomiya, I. Terada and S. Fushida *et al.*, 2004. Thiazolidinedione, a peroxisome proliferator-activated receptor-ã ligand, inhibits growth and metastasis of HT-29 human colon cancer cells through differentiation-promoting effects. Int. J. Oncol., 25: 631-639.