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EpCAM as a novel therapeutic target for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor worldwide. Due to the heterogeneity nature, prognosis for patients with HCC remains unsatisfactory. The conventional treatments like chemotherapy and radiotherpay fails to cure the disease most of the time and this may be due to the presence of cancer stem cells (CSCs). Cancer stem cell is a small population of cancer tissues responsible for chemoresistant, radioresistant, and cancer relapse through various mechanism like ATP binding cassete (ABC) effulus and ALDH inhibitor. Numores cancer stem cell markers are idendified for the liver cancer, such as Epithelial cell adhesion molecule (EpCAM), CD133, CD90, CD13. EpCAM is one of the first tumor-associated antigen and a marker for most epithelial cancers except renal cell carcinoma, urothelial carcinoma and squamous cell carcinoma. Also it is a marker for liver stem cells/progenitor cells. EpCAM plays a major role in cell-cell migration, cell proliferation, tumorogenisity, metastasis. Also, it acts as a potential target for EpCAM positive carcinomas like breast cancer, colon cancer and liver cancer. This entire review deals about how EpCAM can be used in the near future as a potential therapeutic target for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one among the leading cause of cancer death in many countries. The prevalence of HCC has been increasing in Asia-Pacific region, including Australia, New Zealand, and India over the past several decades. Cancer Registries from five Indian urban populations (Mumbai, Bangalore, Chennai, Delhi, and Bhopal) revealed that, liver cancer ranks as the fifth most common cancer among both male and female. As reported by a study cohort of 213 HCC patients from 1999 to 2005, the incidence of HCC is higher in men (83.1%) than in women.[1] In India, HBV infection, HCV infection and alcohol consumption are the main causes of HCC.[2] Major etiologic agents in HCC are chronic viral infections such as hepatitis B & C, factors like chronic alcoholism and metabolic disorders also minimally involved in HCC.[3] Surgery, chemotherapy and radiotherapy are the standard treatment options for HCC. To date, Sorafenib, a Multikinase inhibitor is the only drug approved by FDA for liver cancer.[4] Most of the conventional treatment fails to eradicate the tumor because of the cancer stem cells (CSC).[5] CSC has the tendency to self renew, can differentiate into multiple lineage, resistant to chemotherapy and radiotherapy through various other mechanism like ATP binding cassette efflux, ALDH inhibitor.[6,7] Eradicating CSC by competent targeting agents may have the potential to cure HCC without any relapse. Epithelial cell adhesion molecule (EpCAM) also known as CD326, C017-1A, GA733-2, TACSTD1, KSA, EGP40, HEA125, MK-1, EGP-2, EGP-34, ESA and KS1/4 was initially identified as a tumour associated antigen for several carcinomas of different origins in 1979.[8] then further reports established that, EpCAM can serve as a novel marker for liver cancer stem cells.[9] It has been shown to express on the basolateral cell surface of most of the epithelial tissues except squamous epithelia, epidermal keratinocytes, gastric parietal cells, myoepithelial cells, thymic cortical epithelium and hepatocytes.[10] EpCAM is over expressed in majority of human epithelial carcinomas including colorectal, breast, prostate, head and neck, and hepatic carcinomas.[11] Mostly, it is actively involved in proliferation and maturation of both normal and neoplastic tissues.[12] In addition, EpCAM is being used as a target for immunotherapy of certain human epithelial cell carcinomas.[13,14] Immunohistochemical analysis of EpCAM will help us to know the progress of cancer development and for the clinicians, to initiate the therapy which is suitable for patients with EpCAM positive liver diseases.[15] This review explains in detail about EPCAM structure, liver disease, cancer stem cell and

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various approaches to target EpCAM for HCC therapy.

2. Structure of EpCAM

EpCAM is a type I trans-membrane glycoprotein with a molecular weight of more than 40,000 Da.[16] and is structurally not related to any of the four major families of cell adhesion molecules.[17] It is encoded by the GA733-2 gene located on the long arm of chromosome 4.[18] This protein comprises of 314 amino acids of extracellular, trans-membrane and cytoplasmic domains. The extracellular domain of EpCAM (EpEX) is a 242 amino acid long with 12 cysteine residues and several glycosylation sites. The EpEX further divided as 3 sub domains. The first sub domain contain two epithelial growth factor sites, the second sub domain contains thyroglobulin, IGF binding proteins 1 and 6, the third sub domain is cysteine free portion.[19] The small trans-membrane domain comprised of 21 amino acids. Short intracellular domain (EpICD) comprises 26 amino acids with two α actinin and tyrosine phosphorylation binding sites (see Fig. 1).[20]

3. Functions of EpCAM

The role of EpCAM is not only limited to cell-cell adhesion as the name indicates, but also includes cell migration, proliferation, cell cycle metabolism, cell signaling, cell differentiation, metastasis, regeneration and organogenesis of liver. These functions are performed by EpCAM alone or combined with several specific proteins. An EpCAM play a role in cell-cell adhesion via intercellular homophilic interactions, by its extracellular domain contains EGF-like domain and a thyroglobulin domain. In addition, the same function can also be performed with the help of claudin 7 proteins.[21] Cell migration is reduced by inhibition of cell-cell adhesion.[22] EpCAM along with D5.7A proteins can activate cell proliferation.[23] Cell cycle can be activated by the intracellular domain of EPCAM.[24] EpCAM along with CD44v4-V7 and D5.7A activates metastasis in malignant tumour (see Fig. 2).[25,26]

4. EpCAM signaling pathway

The EpCAM signaling pathway can be activated by intramembrane proteolysis and shedding of the extracellular domain of EpCAM.[27] EpCAM was sequentially cleaved by two important proteins named as tumour necrosis factor alpha converting enzyme (TACE) and peresenilin 2(PS-2) as EpEX and EpICD. EpEX is released out of the cell, whereas EpICD is released into the cytoplasm.[24] Four and one-half LIM domain protein 2 (FHL2) is a protein which contain two binding sites such as EpCAM and β-Catenin. FHL2 is identified as cytosolic interaction partner for EpICD.,[28] and also it regulates the TACE and PS-2 protein activities.[29] Simultaneously the Wnt signaling pathway activated by the binding of Wnt ligand with its receptor such as frizzled and LRP 5/6, recruits disheveled and induce β-Catenin degradation complex (AXIN, APC, GSK3). This complex inhibits the phosphorylation of β-Catenin. Therefore the β-Catenin gets accumulated in the cytoplasm.[30,31] This

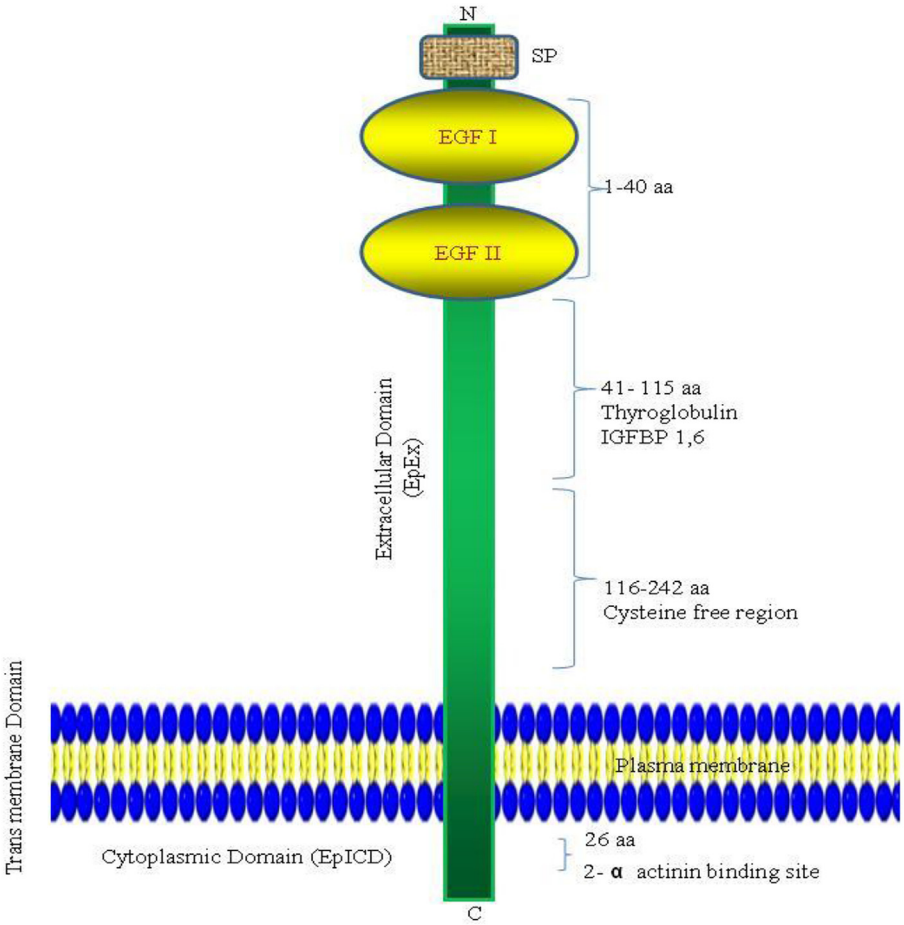

Fig. 1. Schematic diagram of EpCAM structure consist of Extracellular domains (EpEX), transmembrane domain, intracellular domain (EpICD): EpEX starts with signal peptide (SP), EGF like domain repeats, Tyroglobulin, IGF binding portion and cysteine poor region. EpICD contain two α-actinin binding sites.

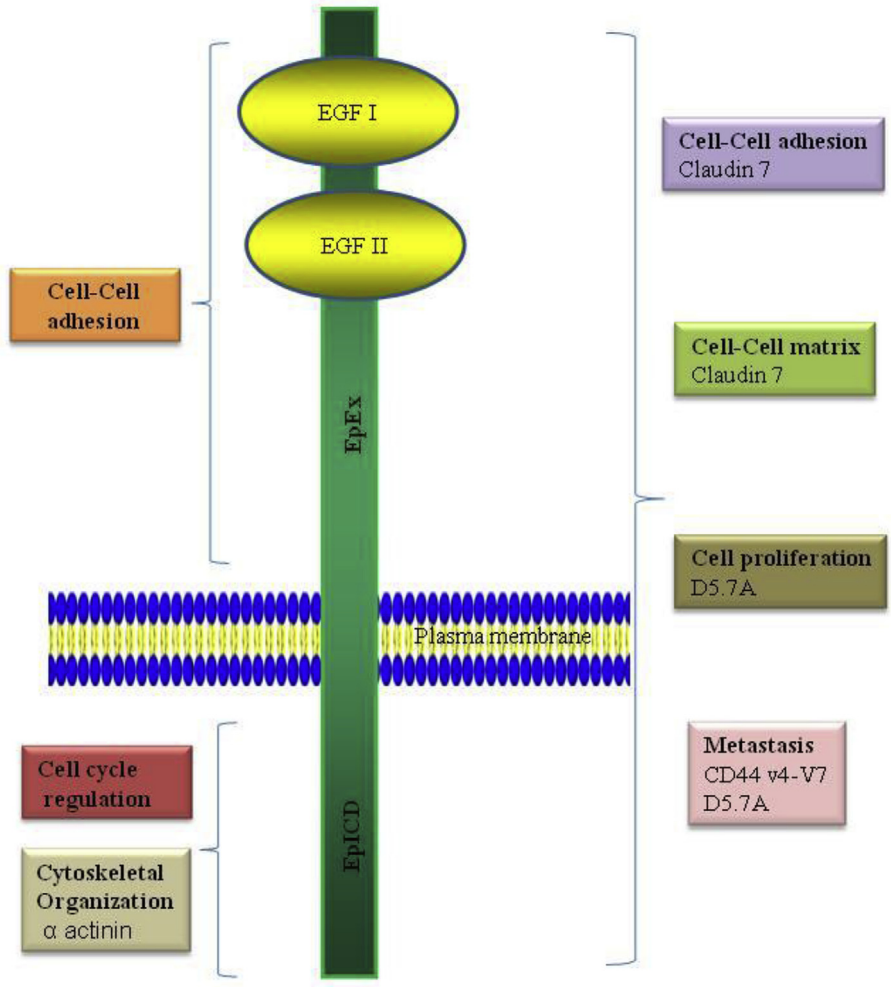

Fig. 2. EpCAM function: EpCAM alone or along with specific proteins involved in several functions including cell-cell adhesion, cell-matrix adhesion, cell proliferation, cell cycle regulation, cytoskeletal organization and metastasis.

accumulated β-Catenin binds with FHL2 and EpICD complex and translocates into the nucleus. The large nuclear complex proteins regulate gene transcription and activate the EpCAM target genes such as c-myc cyclins, and TCF1 (see Fig. 3).[13,24]

5. EpCAM in liver diseases

EpCAM also called as TACSTD1 (tumour-associated calcium signal transducer protein 1-precursor) and is a marker for most epithelial cancer.[8] High expression of EpCAM in chronic carcinomas indicates a poor prognosis.[11] In liver diseases, EpCAM was highly expressed in biliary cell related diseases such as cholangiocarcinoma (CHC), biliary cirrhosis, metaplasia, and biliary atresia.[32,33] But it is not expressed in hepatocyte related diseases such as adenomas and some of the hepatocellular carcinomas (HCC). However, most of the HCC showed expression of EpCAM.[34–36] Taro Yamashita & Yoon SM explained that EpCAM positive HCC has unlimited cell proliferation and long telomerase length, whereas, EpCAM negative HCC has limited proliferation and short telomerase length.[36,37]

6. EpCAM as cancer stem cell marker

Cancer stem cells or tumor initiating cells are a small population in a tumor tissue, which have the stem cell features like self renewal and can differentiate in to numerous cell types.[38] Apart from this, CSC is responsible for extensive proliferation. Failure of many treatment protocols such as chemotherapy, radiotherapy by ATP binding cassette (ABC) efflux mechanism, aldehyde dehydrogenase (ALDH1) inhibition, enhanced DNA repair, inhibition of apoptosis.[39] results in tumor relapse. CSC is identified by unique surface markers and by nature of form spheroids. Atsushi Takai and his group demonstrate EpCAM is a stem cell marker which expresses high stemness, and they also claimed wnt-β catenin is an essential pathway required to maintain the stemness in HCC.[40] Taro Yamashita et al., observed that EpCAM positive HCC show poor prognosis and chances of getting tumor relapse even after standard chemotherapy.[37] Chen Y et al., studied EpCAM negative and EpCAM positive HCC cells. They found that EpCAM negative cells are unable to form spheroids in culture, sensitive to chemotherapy and showed less/no tumorogenisity in vivo, but EpCAM positive HCC cells can cause invasive tumors in NOD/SCID mice, forms numerous spheroid formations in vitro, and resistant to standard chemotherapies like 5-FU, cisplatin and Doxorubicin.[41]

7. EpCAM role in therapeutics

Recent studies confirm that, EpCAM can be used as a cancer

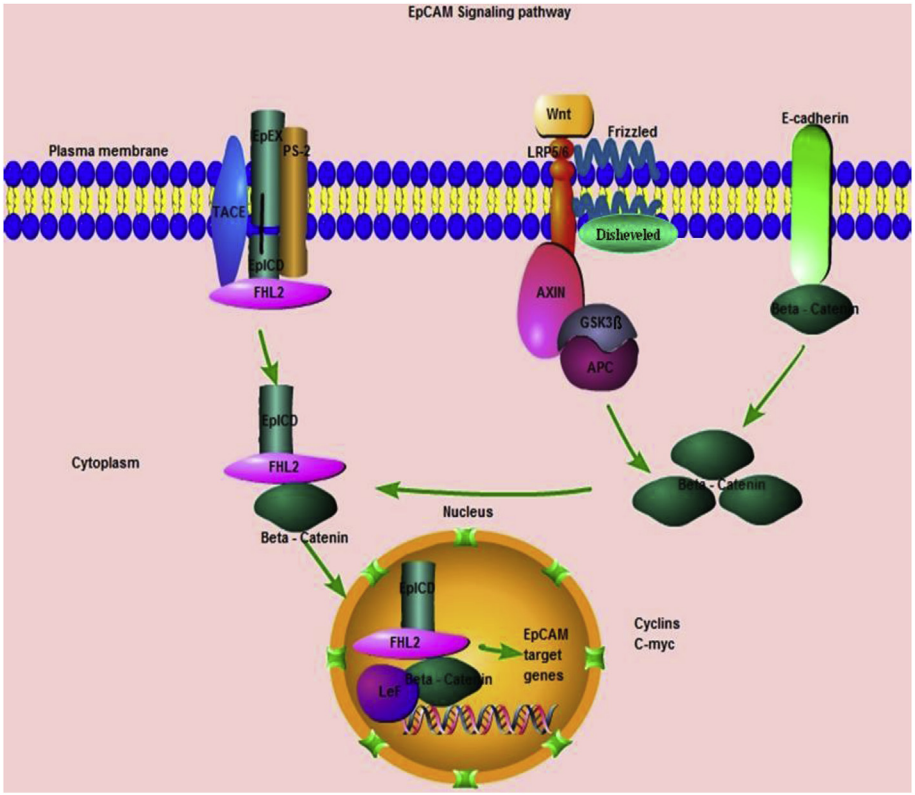

Fig. 3. Schematic representation of EpCAM signaling pathway: Intra cellular domain of EpCAM (EpICD) cleaved by TACE and PS-2 enzymes and translocate into the cytoplasm. Meanwhile, β- Catenin accumulates in cytoplasm due to the inhibition of β- Catenin degradation complex (AXIN, GSK3 β, APC) in Wnt - β Catenin pathway. With help of FHL2, EpICD and β Catenin enters into the nucleus. These nuclear complex proteins regulate gene transcription and activate the EpCAM target gene such as Cyclins and C-myc.

stem cell marker and a potential therapeutic target for EpCAM positive tumors. Targeting EpCAM by gene silencing, inhibiting wnt −β catenin signaling, vaccine, inducing nanomedicine approach and specific monoclonal antibodies, etc., can eradicate tumors without any relapse.

8. Knockdown of EpCAM gene

Gene silencing is a techniques used to knockdown the desired gene by using RNAi (siRNA) for inhibiting the particular gene function. EpCAM is a marker of many carcinomas and also a cancer stem cells involved in variety of functions like cell proliferation, cell migration, invasion, metastasis, chemoresistant and tumor relapse. So, silencing EpCAM gene can help to work better with conventional chemotherapy without any influence of cancer stem cell activity. Knockdown of EpCAM in different EpCAM positive cell lines show suppressed proliferation, reduced spheroid formation and enhanced chemo and radio sensitivity.[42] Yan Li et al. and his group conformed that EpCAM gene silenced HCC cell lines were sensitive for doxorubicin, cisplatin and 5-FU.[43] Bae et al., have reported, gene silencing of EpCAM in HCC showed less tumourogenesis, and no spheroid formation in vitro.[44] Gao J. and his team found EpCAM gene silenced cell line displayed increased apoptosis, inhibition of cell proliferation and increased cytotoxic effect of 5-FU.[45] Madhu beta and his colleagues observed, silencing of EPCAM effectively reduces the oncogenic miR-17-92 clusters.[46] The enhanced cytotoxic effect of chemotherapy achieved in EpCAM knock down by down regulating anti-apoptotic protein Bcl2 and up regulating pro-apoptotic protein Bax, via the ERK1/2 and JNK, MAPK signaling pathways.[47]

9. Wnt/β-catenin signaling inhibition

Wnt/β-catenin signaling is an essential pathway for maintain stemness in both normal liver as well as liver carcinomas. Especially in HCC, Wnt/β-catenin signaling play major role in spheroid formation as well as maintenance of acinous. Atsushi Takai et al., proved that EpCAM positive cells are more sensitive for TGF/β-induced epithelial-mesenchymal transition with highly tumorigenic and metastatic potential in vivo.[40] In 2007, Taro Yamashita et al., observed, inhibition of Tcf/β-catenin complex can reduce EpCAM expression in both normal hepatocyte and HCC.[13] Curtis J. Henrich and his colleauges conformed by flow cytometry, wnt-β-catenin signaling inhibition can decrease EpCAM expression.[48] Anja Lachenmayer et al., noted that inhibition of wnt signaling in EpCAM positive HCC decreased tumor growth and increased survival rate of standard chemotherapeutic treatment in vivo.[49] Interestingly Fako V et al. claimed that Pimozide (PMZ), an antipsychotic drug has effectively inhibited the EpCAM positive HCC cell growth through disturbing the wnt/β-catenin pathway.[50]

10. Monoclonal antibody

Cytotoxic lymphocyte of the innate and adaptive immune system, including cytotoxic T cells, macrophage, and nature killer cells play an important role against tumors. Accordingly, Bi-TE, trifunctional Monoclonal antibody have more attention in cancer therapy. 17-1A is the first EpCAM directed monoclonal antibody applied for the cancer therapy of gastrointestinal tumors.[51] Later in 2009, catumaxomab, a tri-functional bi-specific monoclonal antibody was accepted for treating EpCAM positive tumors.[52] Followed by many drugs like edrecolomab, Adecatumumab (MT201), and EMD

273066 invented for the therapy of EpCAM positive carcinomas. Catumaxomab is one of the most widely used drugs against epithelial cancer. Initially this drug has been developed for the management of the complications associated with advanced cancer, such as malignant pleural effusions and ascites but recent data supports the use of Catumaxomab for the treatment of EpCAM positive tumors. Catumaxomab is produced via quadroma technology and consists of mouse IgG2a and rat IgG2b. Mouse IgG2a antigen binding site recognize EpCAM positive tumour cells, rat IgG2b binding site recognize the T cell and Fc portion bind the Fc gamma receptor type I, type II and type III positive cells. The action of Catumaxomab on EpCAM positive cancer cells start by activating ADCC phagocytosis from the FC region, apoptosis by T cells & FC γ receptor activating cytokines.[53]

11. Immunotoxin

Viventia Bio Inc, developed a novel EpCAM specific immnotoxin VB4-845 for EpCAM specific tumors. Ogawa K et al., claimed that in combination of VB4-845 and 5-FU showed strong cytotoxicity, decreased cancer stem cell population and strong suppressed of spheroids in HCC cell lines whereas in single 5-FU treatment arm failed to show cytotoxicity, expressed increased cancer stem cell population and incapability of affecting spheroid formation.[54] Lv M and his group constructed an EpCAM targeted immunotoxin scFv2A9-PE or APE. They were conformed the anti tumor activity of scFv2A9-PE or APE immunotoxin by reduction of EpCAM positive HCC cell viability in MTT, and immunotoxin localized to endoplasmic reticulum 24 h later in Immunofluorescence.[55]

12. Micro RNA specific therapy

Therapies, those targeting cancer stem cells could be an excellent choice to eradicate the tumor population rather than targeting only cancer cells. The liver cancer stem cell marker EpCAM also acts as a liver stem cell marker. So, Targeting EpCAM in HCC can lead to affect the liver stem cell too. Ji J and his college found miR-155 is a specific micro RNA highly elevated in only EpCAM positive HCC not in EpCAM negative HCC, liver stem cells, fetal liver, and adult liver. So, targeting EpCAM specific mir-155 has excellent method to eradicate the CSC without affecting liver stem cells.[56]

13. Conclusion

Standard conventional chemotherapy fails to eradicate the tumor due to the involvement of CSC. As we have discussed earlier, CSCs in the tumor are prone to metastatic and drug/radiation resistant than non-CSCs, and also liable for cancer relapse hence tumor remission warrant the development of new markers that can specifically eradicate CSCs. EpCAM is one of the novel biomarker identified by many studies for liver CSC. It is involved in all stages of tumor development such as invasion, proliferation including metastasis by wnt-β-catenin, and other signaling pathways. Hence, targeting EpCAM through monoclonal antibody, EpCAM gene silencing, and inhibition of wnt-β-catenin could have vast therapeutic advantage for the EpCAM positive HCC.

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