Review Article
Advances in malignant pleural mesothelioma therapy: targeting EphA2 a novel approach

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Abstract: Malignant pleural mesothelioma (MPM) is an aggressive neoplasm with a poor prognosis. MPM grows from the mesothelial cells lining the surface of the lung and chest wall called Pleura. Exposure to asbestos is mainly linked to the development of MPM. Approximately 80% of the tumors are pleural in origin, and up to 3000 people are diagnosed with MPM in the United States annually. The incidence of MPM is expected to rise in the coming decades particularly in the developing countries. Although there is an increase in the awareness of danger associated with the use of asbestos, its use is still prevalent in Australia and Asia because of its durability and low cost. This further warns and adds to the mortality and morbidity of patients with MPM globally. The traditional treatment strategies have shown only modest improvement towards the disease. MPM is difficult to treat because of the fact that the time between the exposure to asbestos and the appearance of symptoms is extremely delayed, and also due to tumor involvement with the pleural surface and the adjoining tissues such as the chest wall, pericardium and sub-diaphragmatic organs. Despite advances in the diagnostic and treatment approaches the median survival rate for MPM is between 9 to 17 months. The standard care with double agent has shown modest improvement however, multimodality approach using novel targets may have potential to achieve the improvement in the survival rate. In this review we give an update on the conventional treatment modalities and discuss about various molecular targets including receptor EphA2, a novel target gene which may be considered as a biomarker for the diagnosis and treatment of MPM.

Keywords: Receptor EphA2, EphrinA1, malignant pleural mesothelioma, receptor tyrosine kinases, asbestos, surgery, chemotherapy, gene therapy

Introduction

Although 50 years have been passed since the discovery of first incidence of MPM, treatment of this neoplasm continues to be a challenge till to date. The diagnosis of patients with MPM remains extremely poor despite several advancements in the strategies and techniques. MPM is an aggressive tumor of the pleura associated with asbestos exposure. Besides asbestos exposure, radiation, simian virus 40 and genetic predisposition to mineral fiber are also considered as causative agents [1-3]. The incidence of MPM is expected to peak globally particularly in Europe and the other developing countries [4-6]. In Western Europe more than 5,000 new cases are diagnosed annually and a death toll of more than quarter million is expected in next 40 years [7]. In Japan peak inci-

dence of death is expected due to MPM in 2025 [8]. In Australia highest incidence of MPM have been reported and the incidence continues to rise with expected peak in 2021 [9]. In India, China, Indonesia and Vietnam since the use of asbestos containing material is still very common, it is predicted that the global burden of MPM is expected to mount in the coming decades [5, 10].

MPM is generally a disease of advance age, the median age at diagnosis ranges from 45 to 85 years in the United States. Because of occupational exposure the incidence is more in men than women (5:1 ratio). MPM is difficult to diagnose and often requires experienced pathologist to differentiate MPM from other benign tumors. MPM is classified into 3 major sub-types based on histology: epithelioid, sarcomatoid,

and mixed or biphasic. The epithelioid tumors are most common type and represents of 50% to 70% of all MPM diagnosed. The epithelioid tumor cells are usually more uniform, cuboidal and have easily identifiable nuclei. It is least aggressive and responds to the treatment better compared to sarcomatoid and mixed phenotypes [11]. The sarcomatoid tumors are less common, and it comprises of 7% to 20% of cases diagnosed. It is the most aggressive subtype and most difficult to treat. Normally the cells are spindle shaped with elongated nuclei. Whereas, the mixed subtype as the name implies consists of the combination of both the epithelial and sarcomatoid cells. The mixed subtype makes up about 20% to 35% of mesothelioma cases [8, 11]. The treatment options for MPM depend on the stage of the cancer early or advanced and the patient's age. The options available are: Surgical resection along with, chemotherapy, radiation therapy, immunotherapy, and gene therapy. However, limitation to the studies such as small sample size, nonrandomized trial, patient selection bias etc. always exits and would have potential effect on the outcome of studies.

Surgery

Surgical treatment for MPM has been controversial. Pleurectomy or palliation surgery is considered as the best option for the treatment of early stage disease. MPM is associated with the development of pleural effusions and palliation is recommended to relieve the symptoms such as dyspnea and chest pain. A common palliation therapy is insufflation of talc as a sclerosing agent into the pleural space to obliterate the pleural cavity in order to prevent reaccumulation of pleural fluid. In some cases talc pleurodesis is combined with video assisted thoracoscopic surgery (VATS) to debulk/decortications of the pleural tumor tissue to control the symptoms with minimal mortality [12, 13]. Radical resection or extrapleural pneumonectomy (EPP) is used at some centers, for the localized disease control but it cannot influence the distant recurrence [14]. EPP have shown improved mortality and morbidity [15, 16]. Surgery alone cannot guarantee the complete resection of tumor suggesting MPM progress in spite of local control. About 54% recurrences have been noticed after EPP at the median interval of 19 months [17, 18]. Albeit surgical techniques currently used to debulk the tumor have been dramatically improved but the

treatment of MPM is still difficult. At the main centers, EPP or pleurectomy along with adjunctive therapies such as chemotherapy, immunotherapy and radiotherapy is most commonly performed for MPM patients for the long-term survival benefit and with acceptable rate of morbidity and mortality [19].

Chemotherapy

Chemotherapy regimens used against MPM has not proven effective till to date because MPM is often resistant to chemotherapy. Chemotherapy in mesothelioma patients is as an option for both patients with unresectable tumors and for patients with resectable tumors. Chemotherapy is provided as single agent or in most of the cases, combination of chemotherapy with neoadjuvent or adjuvant is recommended. Drugs used for the treatment of MPM are classified as: 1) Alkylation agents; 2) Anti-metabolites; 3) Anthracyclines; 4) Platinum Compounds; and 5) Plant alkaloids. Alkylating agents are the earliest chemotherapeutic drugs used to treat cancer. Cyclophosphamide, Ifosfamide, Mitomycin, Mechlorethamine, Thiotepa, and Meiphalan are among the drugs commonly used for treating solid tumors [20-22]. These drugs work directly on DNA and prevent cell division process by cross-linking the DNA strands and causing abnormal base pairing. However, these drugs are toxic and affects cardiac and renal functions and long term use may cause secondary cancers [23, 24]. Combination of Cyclophosphamide with cisplatin was well tolerated for metastatic MPM however; the survival rate was not satisfactory [21].

Anti-metabolites, such as Gemcitabine and Pemetrexed are called folate based drugs. Pemetrexed inhibits the function of enzymes used in purine and pyrimidine synthesis, thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). Pemetrexed prevents the formation of DNA and RNA by inhibiting the formation of precursor for purine and pyrimidine, which are required for the growth and survival of both normal cells and cancer cells [25]. The median survival is 10.3 months after the treatment [26]. Low blood cell counts, mental fatigue, nausea, vomiting, and oral mucositis were noted in patients treated with folate based drugs [27]. In a phase III trial, Pemetrexed plus Cisplatin showed improved survival compared to Cisplatin alone for patients with MPM [28].

Anthracycline drugs, such as Doxorubicin, Epirubicin, and Pirubicin are known to interact with DNA by intercalation and inhibition of macromolecular biosynthesis. These drugs act as anti-tumor agents by inhibiting the progression of the enzyme topoisomerase-II, which relaxes super coils in DNA for transcription. Doxorubicin stabilizes the topoisomerase-II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being re-sealed and thereby stopping the process of replication in cancer cells [29, 30]. In a phase II trial, combination of Doxorubicin and Mitomycin gave a superior response when compared to Doxorubicin and Cisplatin in phase-II study [31], and about 29% of improved response was observed. *In vitro* studies with mesothelioma cell line showed synergistic effect with combination of Mitomycin and Cisplatin. Combined Cisplatin and Gemcitabine phase-II trial for advanced MPM resulted in the improvement of the symptoms in the patients who responded to the treatment [32-34]. A multi-center phase-II study used two active regimens in a sequential order as a first line chemotherapy for unresectable MPM. The regimens included Cisplatin, Gemcitabine followed by Mitoxanthrone/Methotrexate/Mitomycin. This trial yielded good results, disease control with improved morbidity [35]. The toxicities were restricted to hematological parameters such as neutropenia, anemia, thrombocytopenia, and vomiting.

Platinum based drugs includes, Cisplatin, Carboplatin, and Oxaliplatin [36]. These drugs bind to DNA of the neoplastic cell and induce programmed cell death. The binding affects both replication and transcription of DNA, as well as mechanisms of DNA repair. Chemotherapy with dual agents produced higher response rate compared to single agent therapy. The combination of cisplatin with pemetrexed yielded significantly longer overall survival (12.1 verses 9.3 months) in a clinical trial of 456 MPM patients with improved quality of life [33, 37]. In addition, patients who received Folate and vitamin B12 supplement had improved survival rate, and lesser toxicity [38]. Moreover greater mean number of cycles of therapy were performed on the patients when compared to group that did not receive supplements. This regimen has become the standard of care and the first line treatment for MPM in advance stage [28]. Nephrotoxicity, neurotoxicity, nausea, vomiting, and electrolyte disturbance were some of the toxic effects noticed with cisplatin based drugs [39]. Plant alkaloids, drugs such as Paclitaxel, Docetaxel are mitotic inhibitors, that affect the formation of microtubules during cell division between metaphase and anaphase, preventing further cancer cell progeny [40]. Paclitaxel also induces programmed cell death or apoptosis [41]. Single agent therapy had limited benefits for the MPM patients. *In vitro* studies on MPM cell lines with Docetaxel and Paclitaxel indicated that these drugs induced DNA single-strands breaks and cell death [42]. A phase-II trial with Docetaxel produced disappointing results [43, 44]. However, a randomized trial conducted for five years using trimodality chemotherapy (Paclitaxel + Carboplatin) and radiotherapy approach became an acceptable option for the patients for MPM for early stage tumors [45]. The combination of Docetaxel with CPT-11 for stage III- IV MPM patients showed survival of 8.5 months, and the toxicity was noticed in 7 of 15 patients along with neutropenic fever, and diarrhea [46].

Radiation therapy

The treatment of MPM by radiotherapy has been limited, because of its diffuse nature of expansion on the pleural surface. The sensitivity of MPM to radiotherapy has been very modest and the effective doses used would harm the adjacent vital tissues [47]. However, the postsurgical radiotherapy has been proven effective, as it prevented tumor cell seeding at the wound site [48]. The most recent and effective radiotherapy technique used is the intensity modulated radiotherapy (IMRT), in patients who underwent extra pleural pneumonectomy [49]. IMRT might have the potential to cover the dose related concerns and this has been applied with no toxicity in patients with hemithorax [50]. The combined use of radiotherapy and extra pleural pneumonectomy in clinical practice have proven successful to manage local tumor however, patients tend to die with metastatic disease. Hence the outcome of radiation therapy has been disappointment in the management of MPM.

Immunotherapy

MPM patients have been subjected to immunotherapy with limited success. Several animal studies were conducted and large body of evidence indicates that immunotherapy resulted in

substantial tumor regression in mouse models of MPM [51, 52]. We earlier reported that antibodies to Interleukin-8 (IL-8) significantly inhibited the tumor growth in a nude mouse model of MPM [53]. The administration of Interleukine-2 showed significant reduction in tumor size and improved survival rate at the initial stage in a mouse model of MPM. However, at the late stage with larger tumors IL-2 failed to inhibit tumor growth [54]. The cytokine therapy involving interleukin-2 or interferon gamma induce a weak response in patients with early stage of disease however, the therapy failed in advanced stage disease of MPM [55, 56]. IFN-α/β induce anti-proliferative effect in tumor cells and modulates CD4+ and CD8+ T cells during initial phase of antigen recognition [57]. A continuous delivery of recombinant granulocyte-macrophage colony stimulating factor (GM-CSF) induced several part responses despite procedure related difficulties [58]. Immuno-modulatory antibodies against B7-H3 a member of B7 family showed promising response in epithelial phenotype [59]. The combination of immunotherapy (IFN-γ) with chemotherapy (Methotrexate) for stage-I and -III disease has shown promising results, with a median survival rate of 17 months and acceptable toxicity [60]. Although, moderate success have been achieved with immunotherapy in management of MPM patients, it is possible that in future certain target cytokine or chemokines may emerge as an effective candidate against MPM and may be included in the standard of care procedures list.

Mesothelioma biomarkers

Till to date, the prognosis of MPM is extremely poor. The available treatment strategies are limited. Identification of a biomarker for the diagnosis may help improve the diagnosis and benefit MPM patient's therapy. Several proteins have been designated as biomarkers for the diagnosis of MPM (**Table 1**).

Targeted therapy

Conventional therapies have failed to show the improvement in survival of the MPM patients. Treatment remains toxic affecting normal tissues along with tumor tissue. Besides, the selection of patients at appropriate stage of the disease is not easy. Recently several novel biomarkers have been identified which are expressed mostly by tumor tissue and not being expressed by the surrounding normal tissue. The expanding knowledge on molecular mechanisms of the development of mesothelioma and the newer technologies helped identify the novel biomarkers which are considered as potential candidates for MPM therapy are discussed.

The epidermal growth factor receptor

Epidermal growth factor (EGFR) belongs to the ErbB family that has been found to be over expressed in MPM. The activation of EGFR promotes proliferation, cell survival and transformation. Approximately, 60% of the 80% of MM patients express EGFR, yet attempts to use EGFR as a prognostic marker have been futile. A phase-II trial of an EGFR inhibitor, Gefitinib, was conducted on small group of MPM patients revealed no correlation between the expression of receptor and the response to the drug in those patients [61]. Although Gefitinib has showed inhibitory effect on mesothelioma cell lines but the clinical studies on MPM patients showed limited efficacy of the drug suggesting single agent treatment is unsuccessful [62]. Recent report on mutations of EGFR tyrosine kinase of MM patients by Foster, et., al., indicates that 31% of patients had mutations (mut+) and the remaining were wild type (mut-) suggests that these mutations were predictive for optimal resectability [63]. The group of patients with mut+ may be better responsive to TK-inhibitor therapy. However, this needs to be confirmed by future studies.

Met as a target for MPM

Receptor tyrosine kinases (RTKs) are the high - affinity cell surface receptors with a similar structure which have roles in both normal development and tumorigenesis. The widespread deregulation of RTKs in cancer makes them potential therapeutic targets for a variety of malignancies [64]. The receptor for hepatocyte growth factor (HGF), a transmembrane receptor tyrosine kinase, constituting a 145-кDa β-subunit and a 50-кDa α-subunit is the Met proto-oncogene product. It controls epithelial growth and remodeling through the coordination of cell proliferation, motility, cell migration, and invasion [65] Met signaling pathway has been found to be aberrantly activated as a result of gene mutations, and over expression or structural rearrangements [66, 67] promotes tumorigene-

Table 1. Malignant mesothelioma markers

1	AUA1	An antibody with epithelial specificity, against a panel of five cultured human meso-thelial cell lines and seven human epithelial ovarian cancer cell lines [101].
2	Carcinoembryonic antigen (CEA)	A glycoprotein involved in cell Adhesion [102]. Absence of CEA distinguishes MM from lung cancer.
3	D2-40	A commercially available monoclonal antibody directed against M2A antigen, a sur-face sialoglycoprotein originally detected in association with germ cell was positive for 96% of MM which indicates the sensitivity for the cells of mesothelial origin [103].
4	Desmin	An intermediate filament present in smooth and striated muscle. Three studies of effusion specimens have demonstrated the value of Desmin in the differentiation of both benign and malignant mesothelial cells from carcinoma cells [104].
5	Hyaluronan	An anionic, non sulfated glycosaminoglycan distributed widely throughout connective and epithelial tissues, known to be produced by mesothelial cells. It is a peritoneal fluid marker of mesothelial cells [105].
6	MCp130	A 130-kDa protein, is reactive with mesothelial cells and useful in the diagnosis of epithelial mesotheliomas [106].
7	Mesothelin	A 40kDa protein present on normal mesothelial cells and over-expressed in several human tumors as a tumor differentiation antigen [107, 108].
8	N-cadherin	A class of type 1 transmembrane proteins which plays important roles in cell adhe-sions. 78% of malignant mesothelioma cases express N-cadherin [109].
9	Protein Phosphatase Inhibitor-1 (I-1)	A mesothelial marker which is an endogenous inhibitor of protein phosphatase-1, involved in signal transduction [110].
10	Thrombomodulin	A sensitive mesothelial marker and is sufficiently specific to be a useful discrimina-tor, positively identifying, the mesothelial nature of a cell population [111].
11	Vimentin	A member of the intermediate filament family of proteins that is especially found in connective tissue as an aid in the identification of cells of mesothelial origin [112].
12	WT1 (Wilms' tumor susceptibility gene)	This protein transcribes during the transition of mesenchyme to epithelial tissues. Continuous expression of WT1 through adult life has been described only in the gonads and mesothelial tissues, and it has been suggested as a marker for meso-thelial lineage [113].
13	Receptor EphA2	A 120KDa protein, member of RTKs family is over expressed in MPM cells and nor-mal mesothelial does not express the receptor. Thus makes it a potential candidate for the diagnosis of malignant from non-malignant tissue of the pleura [93].

sis, especially in the development of invasive and metastasis phenotypes [68].

Met receptor is uniformly expressed in MPM, however a subset of MPMs was reported to carry a point mutation in Met gene, which suggests that Met would be a good candidate RTK for target therapy of MPM [69]. The expression of Met protein has been detected in 74% to 100% of MPM and HGF/SF expression in 40% to 85% of MPM tumor specimens but not in normal mesothelial cells [70]. HGF/Met signaling is involved in MPM growth and also it can change the rate of migration and invasion in a disease that is clinically characterized by local extension. Although, the effects of Met receptor inhibition in preventing MPM growth are limited to a minority of MPM cell lines, that also produce HGF [71]. Selective small molecular inhibitors of

c-Met kinase have been found to induce apoptosis and suppress cell growth both *in vitro* and *in vivo*. It is predictable that clinical trials of Met inhibitors, in MPM would be most effective in patients whose tumors express both Met and HGF [70, 72]. C-Met specific siRNA can successfully inhibit c-Met expression resulting in significant inhibition of cell viability, and further inhibition strategies targeting the receptor will be sufficient and more effective for clinical treatment applications for MPM in the future [45, 73].

Angiogenesis inhibitors

Patients with MPM have high levels of VEGF hence monoclonal antibodies against VEGF were considered. VEGF receptor, platelet-derived growth factor receptor and c-KIT are expressed by MPM. Sorafenib, a potent inhibitor
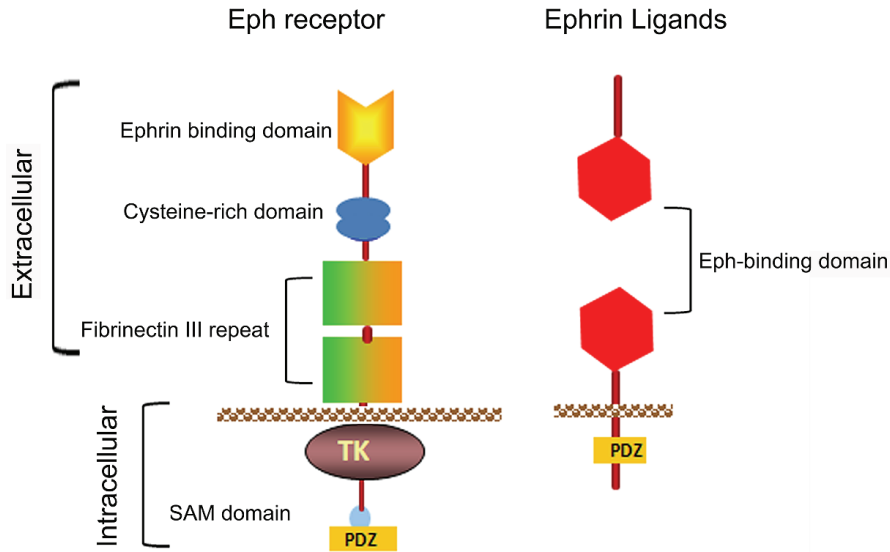
Figure 1. Schematic representation of the domain organization of Eph receptors and Ephrins. SAM = sterile alpha motif; PDZ = PDZ-binding motif; TK = tyrosine kinase domain; GPI = glycosylphosphatidylinositol linkage.

of ras/raf/MEK pathway along with VEGFR and c-Kit was evaluated in unresectable mesothelioma in a phase-II trial with limited response in advanced MPM patients [74], and thus warrants further studies. Other anti-angiogenic inhibitor showed modest improvement and are not recommended for clinical use [75]. To date no clinical trial using anti-angiogenic agents have shown efficacy in improvising response or overall survival improvement for MPM.

Histone deacetylase inhibitors

Histone deacetylases (HDACs) are known to play a critical role in cellular differentiation and malignant transformation of MPM [76]. HDACs manipulate chromatin structure and function of various non-histone proteins and transcription factors. The inhibitor of HDAC showed a partial response against advanced MPM in a phase-I trial [77]. Further clinical studies are warranted before these inhibitors could be recommended as treatment option for MPM.

Receptor EphA2 a novel target

Eph (Erythropoietin-producing human hepatocellular carcinoma) receptors consist of the largest known family of RTKs that plays crucial role in several physiological processes, embryonic development, and progression of various human malignancies [78, 79]. Eph receptors form a cell-cell communication system upon interacting with their ligands called Ephrins. To date, 14 Eph receptors are known and have been divided into two subclasses subclass-A and subclass-B. Eph-A subclass includes 9 receptors which bind with five Ephrin-A ligands. Eph-B subclass includes 5 receptors which bind to three Ephrin-B ligands with the exception of EphA4 and EphB2 receptor which can also bind to Ephrin-Bs and Ephrin-A5 respectively, and the Eph-B4 which preferentially binds to Ephrin-B2 [80, 81]. Ephrin ligands (Eph family receptor interacting proteins) consists of eight members, they are also divided into 2 subclasses -A and -B.

Bidirectional signaling

Eph receptors are transmembrane proteins composed of N-terminal glycosylated ligand-binding domain, a transmembrane region and an intracellular catalytic kinase domain. The cysteine-rich domain facilitates oligomerzation often noticed upon binding with ligands called Ephrins. The intracellular catalytic domain contains highly conserved kinase domain which is involved in phosphorylation of substrates including receptor EphA2 [82]. The sterile a motif (SAM) domain is followed by PDZ binding motif. All Ephrins contain a conserved extracellular receptor binding domain and B-type Ephrins also possess a short but highly conserved kinase domain followed by PDZ binding motif. The Eph signaling is unique in that both receptor and ligand must reorient upon interaction leading to conformational changes which transmits bidirectional signals in both the receptor bearing as well as ligand bearing cells (**Figure 1**). The cells expressing Eph receptors produce
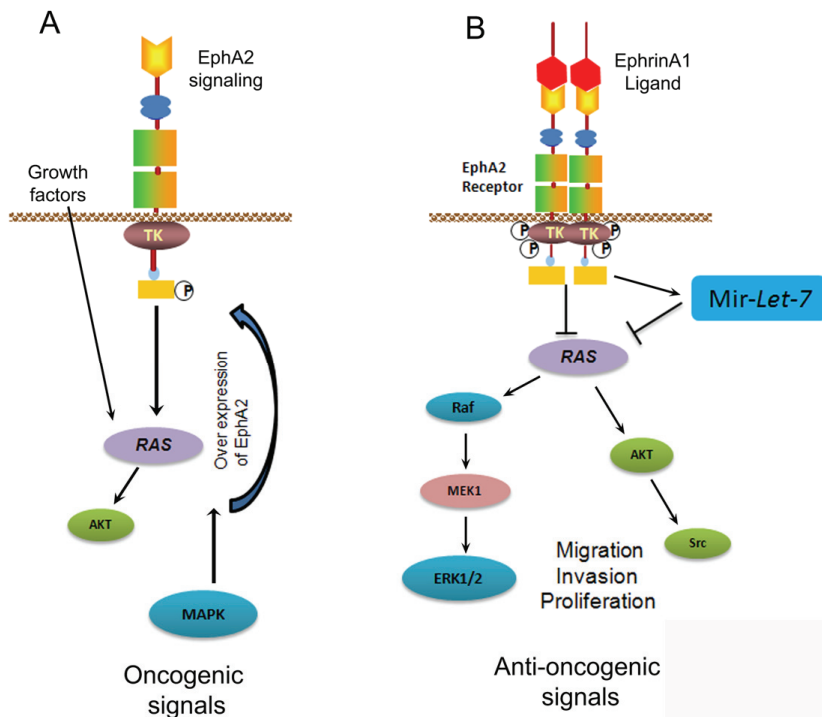
Figure 2. EphA2 signaling pathways in MMC. A. EphA2 signaling promotes oncogenic phenotype. In the absence of ligand, receptor EphA2 kinase function increases by *RAS*, AKT and MAPK activation which promotes malignancy. B. EphrinA1 binding of EphA2 induces tumor suppressive signals. Activation of EphA2 with EphrinA1 leads to phosphorylation of EphA2 and inhibits *RAS*/MAPK pathways. EphrinA1 activation of MMC induced miR-*Let-7a* which targets *RAS* signaling and inhibits oncogenic signals in MMC. Phosphorylation = P; inhibition = ⊥; activation = →.

"Forward" signaling. Whereas the cells expressing Ephrins produces "Reverse" signaling. The specificity of ligand binding is dependent on N-terminal glycosylated domain. The Eph receptors and their ligand Ephrins form heterotetramers upon interactions. Ephrin binding leads to clustering of Eph receptor by the activation of kinase domain, and triggers downstream singling pathways [83].

Expression level of EphA2 receptor activity has a statistically significant involvement in the establishment of carcinogenesis and metastasis through multiple mechanisms [84, 85]. Expression of Eph receptors such as EphA2 is often up regulated in many types of malignancies, yet their exact role in cancer is not well-understood [86]. Studies of Eph receptors in various types of tumors, demonstrate their dual roles in tumor suppression and tumor promotion. In normal cells, binding of Eph2 receptor with EphrinA1 ligand on adjacent cells induces receptor forward signaling, leading to inhibition of Ras/mitogen-activated protein kinase (MAPK) pathway activity, resulting in normal growth. In tumor cells, disruption of cell-cell junctions inhibits EphA2 receptor interaction with endogenous EphrinA1. In addition, Eph receptors are often over expressed and Ephrins are down regulated

[87]. Crosstalk between Eph receptors and other receptor tyrosine kinases such as EGFR (Epidermal Growth Factor receptor) and ErbB2 results in more Ras-MAPK pathway activation, which leads to malignant phenotype [88]. In addition, activation of MAPK signaling activates AKT and promotes receptor EphA2 expression and accumulation in tumor cells (**Figure 2A**). In MM receptor EphA2 is over expressed and it is modulated through the expression of *Ras* oncogene. RTK pathways including EGFR, IGF (Insulin-Growth Factor) and HGF (Hepatocyte Growth Factor) are activated and these RTKs are known to signal through *Ras*, and thus *Ras* signaling pathways play a potential role in MM growth. High levels of EphA2 receptor has been observed in majority of colon cancer samples [89], advanced gastric, ovarian [90, 91] and in non-small cell lung cancers [92]. We reported that MPM cell lines express high levels of receptor EphA2 and normal mesothelial cells did not express these receptors [93]. In addition silencing the receptor with siRNA-EphA2 significantly attenuated the MM growth and induced apoptosis in MPM cells [93, 94]. Furthermore, activation of receptor EphA2 with its ligand EphrinA1 causes phosphorylation of receptor EphA2 and transfer the signal to downstream signaling molecules thereby suppressing tumorogenesis

promoting signals and inhibits tumor growth and migration of MPM cells [95]. However, the molecular mechanisms involved in inhibition of tumor growth not clearly understood. Recently we have also reported a novel mechanism that activation of receptor EphA2 with ligand Ephri- nA1 induces the expression of microRNA *let-7* in MPM cells [96]. Micro-RNA *let-7a* targets *Ras* oncogene which promotes the malignant pheno- type. The expression of *let-7a* may be responsi- ble for the anti-oncogenic responses induced by EphA2 and EphrinA1 interaction in MPM (**Figure 2B**). However, further work needs to be per- formed to understand the molecular mecha- nisms of EphA2-EphrinA1 signaling which could hold potential for future therapeutic strategies for MPM.

We and others have shown that not only ligand activation causes the down regulation of EphA2 by phosphorylation and proteosomal degrada- tion, but also this change in the receptor status correlates with a decrease in anchorage- independent growth and invasion of tumor cells [97, 98]. It suggests that although the causes of EphA2 over expression in most of the aggres- sive tumors remain largely unclear, manipula- tion of EphA2 receptor/EphrinA1 system will aid an opportunity for new therapeutic intervention against MPM. Pasquale et al. and others have highlighted the importance of ephrin-Eph signal- ing in the hindbrain for the establishment of rhombomeres and malignancy [87, 99, 100]. But we are still far from understanding how best the Eph-Ephrin axis can be a target. Future stud- ies on newer strategies of gene therapy target- ing Eph-Ephrin signaling will likely unravel the mechanisms of this unique class of molecules.

Conclusion

Modest success has been achieved so far to- wards the therapy for MPM. Over the past 2 decades the focus of research to find novel tar- gets led to discovery of the association of EphA2 and its ligand EphrinA1 in the develop- ment of malignancy. The mounting evidence from the research involving Eph receptors and their ligands Ephrins puts these molecules as the key players in promoting tumor survival. The mechanisms involved are complex and need further work to fully elucidate their potential in therapeutic strategies against MPM. Now that we know we have identified a target which can be exploited to treat the disease whose cure is

still unsuccessful, MPM. The research strategies should be directed to reap the maximum benefit by targeting the EphA2 receptor. However, fur- ther work with cell lines and animal models should be carried out to understand the func- tions of these complex signaling molecules. EphA2 receptor as a novel target may be a po- tential candidate in future therapies to achieve improved survival benefits for the patients with MPM.

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