Activation of metastatic potential in cancer cells by oncogenes

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ABSTRACT

Cancer disease is a leading cause of death worldwide. The main reason for such high mortality from cancer is due to the highly invasive behavior of cancer cells, which usually results in metastasis. Metastasis is a process by which cancer cells spread to other parts of the body through blood circulation and lymphatic system. Metastasis is an extremely complex process that remains to be a major problem in the management of cancer. Countering metastatic cancer and development of novel with effective therapies were always in the priority list of cancer researchers. Therapeutic goals are the prevention of an initial metastasis in high-risk patients, shrinkage of established lesions and prevention of additional metastases in patients with limited disease. Though many pathways have been hypothesized, regulation of metastasis by oncogenes is considered as an important one. Role of oncogenes in inducing metastasis is becoming stronger day by day. Oncogenes induced replication stress and associated DNA damage results in genomic instability that alter the gene regulation which regulates metastatic potential of cancer cells. Since most of the oncogenes also functions as transcription factors, they are able to modify the genomic regulation in favor of metastasis. In this review we have focused on recent findings about important oncogenes and how they regulate metastasis.

Keywords: Metastasis, Oncogenes, Cancer, Transcription factors.

1. INTRODUCTION

Cancer related deaths are rising worldwide, urging for new therapeutic strategies. Various therapeutic strategies were adopted to overcome the uncontrollable growth of the cancer cells. However, cancer cells always find many ways to become chemotherapy resistant and end up in disease recurrence [1–5]. Countering metastatic cancer and development of novel and effective therapies were always in the priority list of cancer researchers. Several, therapeutic regimes including chemotherapy [6–8] application of toxins obtained from pathogen [9–13], immunotherapies [14–16], and microRNA based therapies [17,18] did not proved enough to reduce the overall outcome of cancer. Especially tumors that are metastasized to different parts of the body is not easy to treat. Metastasis is the process where cancer cells spread to other parts of the body. It can move to the adjacent tissues or to distant tissues or organs. Metastasis is a multi-stage process involving cancer cell motility, intravasation, transit in the blood or lymph, extravasation and growth at a new site [19]. Mechanistically, it can happen by...
invading the nearby normal tissue. It can move into the nearby blood vessels and lymph nodes and travel to other parts of the body and forms tumors there. In due course, it will also initiate angiogenesis to receive the nutrients to grow. Based on its pathological nature, the origin of metastasized tumors can be identified. It is important to note that not all the cells in tumors are capable of metastasizing, because of their tumor heterogeneity [20]. Even though various hypothesis prevails to explain how tumor cells are acquiring metastatic phenotype, one of the substantial theory is the involvement of oncogenes. In this review, we will summarize about recent updates on how various oncogenes regulate/activate metastasis.

2. K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog):

K-Ras is an oncogene, which is highly expressed in many tumors. Basically, K-Ras is a GTPase, which is necessary for various cellular signaling. Colorectal cancer is one of the most significantly affected cancers by K-Ras mutation/activation. Recent study in colorectal cancer patients showed 41% of them had tumors with K-Ras mutation. Further analysis revealed that colorectal cancer patients with K-Ras mutation showed two-fold increase in lung metastasis compared to the colorectal cancer patients without K-Ras mutation [21]. Similarly, another study on colorectal cancer with K-Ras mutation showed increased lung metastasis. Additionally, authors observed that patients with K-Ras mutations were less susceptible to liver and lymph node metastasis compared to patients without K-Ras mutation [22]. This study suggests that organs involved by distant metastasis were different according to the KRAS mutational status in colorectal cancer patients. Recent research identified mTRAIL-R as an important regulator of K-Ras driven metastasis in mouse models. Consistent with this, Human TRAIL-R2 promotes tumor growth, migration, invasion, and metastasis.

3. GLI1 (Glioma associated oncogene 1):

Somatic or germline mutations of GLI1 have been reported in several cancers. Among the three GLIs, GLI1 was found to be the most significant target for cancer therapy, because its activation was found in many cancers by both Hh signaling-dependent canonical and independent non-canonical mechanisms. GLI1 also regulates many oncogenes like K-RAS, C-Myc and AKT, which results in the aggressive and chemoresistant tumors [23,24]. EMT transition is one of the important features for invasion and metastatic transition of primary tumors into a different site. GLI1 is a very potent regulator of EMT transition in different types of cancer including ovarian [25]. Similarly, GLI1 overexpression was found in colorectal tissues compared to the normal colon tissues. Statistical significance was observed between GLI1 overexpression and lymph metastasis of colorectal cancer. This study confirms that GLI1 oncogene is overexpressed in colon cancer and its increased expression correlates with lymph node metastases, T stages and postoperative live metastasis-free survival periods. Another important cancer that is affected by GLI1 overexpression is pancreatic cancer. Many studies have shown that GLI1 is over expressed in pancreatic cancer and positively correlates with invasion and metastasis [26,27].

Increased expression of GLI1 correlates with increased lung metastasis of breast cancer [28]. Further analysis revealed that GLI1 binds directly to chemokine receptors and activates them transcriptionally. Aberrant activation of these receptors results in the ERK phosphorylation and cell migration, preliminary responses of metastasis. Another study showed that GLI1 is overexpressed in triple negative breast cancer cells, an aggressive form of breast cancer which lacks receptor therapy [29]. GLI1 overexpression increased migration and invasion of triple negative breast cancer cell line MDA-MB-231. Further analysis revealed that GLI1 positively regulates MMP-11 (matrix metalloproteinase), protein involved in breaking extracellular matrix during normal physiological processes like embryonic development, reproduction, tissue modelling as well as during cancer cell metastasis. Once again, activation of MMP-11 mediates metastasis through activation of ERK phosphorylation and cell migration as discussed above [30].

4. c-Myc (avian myelocytomatosis virus oncogene cellular homolog):

c-Myc is a transcription factor and an oncogene over expressed in most of the tumors and often results in poor prognosis. c-Myc is also regulated by different oncogenes like GLI1 and etc. The oncogene c-Myc contributes to metastasis of cancer cells by promoting proliferation, inducing genomic instability, angiogenesis and activating EMT (epithelial-mesenchymal transition) pathways [31]. One of the way by which Myc regulates EMT pathways is by promoting TGFβ and SNAIL transcription factor

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Recent study in non-small cell lung cancer shows that over expression of c-Myc is associated with increased metastasis. Further analysis revealed that, c-Myc binds to BCYRN1 (brain cytoplasmic RNA 1, a long non-coding RNA) promoter and activates it transcriptionally. Mechanistically, c-Myc induced activation of BCYRN1 regulates metastasis associated proteins MMP9 and MMP13. Inhibition of c-Myc or BCYRN1 reduces the expression of MMP9 and MMP13 [33]. These results confirm the role of Myc in mediating metastasis in non-small cell lung cancer.

Similarly, a study conducted in ERα positive breast cancer showed increased expression of c-Myc in metastasized lesions. However, increased c-Myc in ERα positive breast cancers does not correlate with ER expression in these recurrent metastatic lesions [34]. Since no correlation was achieved between ER status and c-Myc, authors concluded that combination of hormonal therapy with the strategy to inhibit oncogene c-Myc may prevent growth of ER-positive breast cancer at the site of metastasis.

As we discussed earlier, c-Myc can also be activated by certain transcription factors, like this recent finding where they show that Sterol Regulatory Element-Binding Protein-2 (SREBP-2) transcription factor activates c-Myc and facilitates metastasis in pancreatic cancer model [35]. Activation of SREBP-2 induces c-Myc mediated increase in cancer stem cell properties, protasphere-forming ability and tumor-initiating capability, xenograft tumor growth and metastasis. In colorectal cancer, authors evaluated whether increased amplification or expression of c-Myc oncogene is associated with metastatic phenotype [36]. Interestingly, authors observed that amplification but not overexpression of c-Myc is related to metastatic progression of colorectal cancer. However, over expression of c-Myc is associated with the cancer progression.

5. BCL-2 (B-cell lymphoma 2):

BCL-2 is an anti-apoptotic oncogene that has been found to over express in multiple cancers and mostly in lymphomas [37]. Earlier study in breast cancer model shows the role of BCL-2 in regulating metastasis. In brief, expression of human BCL-2 gene into low bcl-2 expressing human breast cancer cell line MCF7 ADR showed increased lung metastasis compared to vector transfection [38]. BCL-2 was found to regulate metastasis associated gelatinase proteins, which increases the chance of invasion and migration. Another study in pancreatic cancer shows a direct link between BCL-2 and metastasis. In this study, authors expressed BCL-2 in an isogenic panel of pancreatic cell lines which were xenografted into mice and observed for metastasis formation. The results showed increased metastasis which correlates with BCL-2 overexpression. Apart from this, the authors also found apoptotic resistant phenotype in these BCL-2 overexpressed cancers compared to the control cells [39].

A recent study in colorectal cancer has reported about the metastatic activity of BCL-2. HT29 and SW480 colorectal cancer cell lines used in the study were knocked down using BCL-2 siRNA and analyzed for their metastatic potential. Authors have also used siRNAs of different BCL-2 group proteins like MCL-1, BCL-2 and BCL-XL. The results showed that all three proteins, MCL-1, BCL-2 and BCL-XL, have a potential role in the migration and invasion of colorectal cancers independent of their known apoptotic functions. However, among the three proteins, it was found that BCL-2 was a more pronounced metastatic factor [40].

Similarly, the role of BCL-2 in inducing lung cancer metastasis has also been reported [41]. Mechanistically, overexpression of BCL-2 induces the activation of nuclear transcription factor activator-1 family (c-JUN, JunD, c-Fos, FosB and Fra-1 proteins). In turn, these proteins transcriptionally activate the MMP-2 protein. Apart from transcriptional activation, overexpression of BCL-2 activates pro-MMP-2 protein to its active form, thus regulating MMP-2 protein both by transcriptional as well as post-translational mechanisms. The increased expression of MMP-2 enhanced in vivo lung metastasis by 4-fold. Overall, the result showed that BCL-2 promotes tumor invasion and lung metastasis by inducing MMP-2 gene expression. Immunohistochemistry analysis of the normal cervix, chronic cervicitis, cervical intraepithelial neoplasia lesions and cervical cancer showed a significant expression of BCL-2 in cervical cancer tissue compared to the others. The authors also found BCL-2 as a prognostic marker for cervical cancer recurrence and metastasis [42].

6. c-Jun:

c-JUN is another important oncogenic transcriptional factor which has a potential role in metastasis. To analyze the role of c-JUN in breast cancer, c-JUN was overexpressed ectopically in MCF-7 cells. Increased expression of c-JUN resulted in a
significant increase in the S-phase of the cells as well as increased motility and invasiveness. Further analysis revealed that c-JUN overexpressing MCF-7 cells that are transplanted into nude mice via tail vein injection resulted in increased liver metastasis [43]. c-JUN is also found to promote EMT in tumor cells, a first step in the metastatic cascade. Mechanistically, c-JUN which is activated by TGFβ phosphorylates and in turn activates SMAD3 [44,45]. SMAD3 further activates proteins that are involved in EMT. A study in patients with osteosarcoma showed increased pulmonary metastasis. Analyzing the gene expression profile by cDNA microarray in two different human osteosarcoma sublines MNNG/HOS and 143B (both cells differ in spontaneous pulmonary metastatic potential) showed increased expression of MMP-1 in the highly metastatic 143B cells compared to MNNG/HOS. In vitro analysis showed that c-JUN and FRA-1 were phosphorylated and bound to MMP-1 promoter in 143B cells. These results suggest that MMP-1 plays an important role in the metastasis of osteosarcoma which is regulated by c-JUN [46]. Similarly, c-JUN also transcriptionally regulates MMP-1 expression in hepatocellular carcinoma cells [47].

A comparative tumorigenicity study was done in rats and nude mice by injecting cells that are transformed by different oncogenes (c-JUN, Ras, SV40 and BPV1). Interestingly, cells that are transformed with c-JUN showed increased tumorigenesis and metastasis. Similar types of results were observed in Ras-transformed cells. Additionally, c-JUN-transformed cells showed more angiogenesis compared to the other oncogenes. Mechanistically, it was found that c-JUN transformed cells had a low level expression of anti-angiogenic thrombospordin-I and SPARC genes. These results confirmed that c-JUN has high metastatic potential by regulating angiogenic and anti-angiogenic factors in cancers [48]. A very recent study showed that c-JUN is regulated positively by miR-10B. Inhibition of miR-10B in metastatic breast cancer cells reduced the expression of c-JUN, whereas, increased expression of miR-10B resulted in the accumulation of c-JUN in ROC-dependent and MAPK activity independent pathway. Mechanistically, miR-10B induced accumulation of c-JUN decreases the expression of E-cadherin and promotes EMT [49].

7. AKT1 (v-akt murine thymoma viral oncogene homolog 1):

AKT1 is an important serine-threonine kinase which is important for normal cellular signaling. However, aberrant expression of AKT1 oncogene has been reported in various metastatic cancers. aberrantly activated AKT1 kinase facilitates metastasis by inducing EMT phenotype. Phosphorylated/activated AKT1 phosphorylates GSK-3β and initiates its proteolytic degradation, this in turn results in the increased stability of the transcriptional factor SNAIL. It is well known that the transcriptional substrates for SNAIL are the proteins involved in EMT, for eg., E-cadherin. The authors also found that decreased E-cadherin decreases PTEN expression which in turn increases PI3 which further activates AKT1. AKT1 positively activates itself and leads to more metastasis [50].

Molecular analysis of colorectal cancer cells, normal cells, colorectal tissue samples showed increased expression of phospho-AKT1. Further analysis showed increased expression of P-AKT1 correlates positively with cell migration, invasion, cell mobility and metastatic properties. Additionally, it was found that P-AKT1 regulates the expression of transgelin, a protein involved in transformation. Inhibition of AKT1 in ovarian cancer cells downregulates the expression of transgelin and also inhibits migration and invasion [51]. Another important protein that is regulated by AKT1 and also involved in EMT is TWIST [52].

The oncogene AKT1 is kept inactivated by the tumor suppressor protein PTEN. Studies conducted in prostate cancer showed that PTEN deletion results in the subsequent activation of AKT1. Increased expression of AKT1 activates the chemokine CXCL12 and its receptor CXCR4 which promotes cancer metastasis [53]. Similarly, expression of activated AKT1 in mouse melanoma models showed increased metastatic melanomas with lung and brain metastasis. Additionally, silencing of PTEN in the AKT1 overexpressing mouse models further increased the percentage of lung and brain metastasis. These results confirm that activation of AKT1 alone induces melanoma metastasis, however, loss of PTEN further increases the metastatic potential of AKT1 [54].

8. CONCLUSION

Metastasis is considered as the final stage of cancer, where treatment becomes tougher. Role of oncogenes in inducing metastasis is getting stronger day by day. Genomic instability can be induced by DNA damaging agents [55–57] or changes in DNA repair proteins [58,59], bystander effects [60–63], activation of oncogenes [64,65], failure of tumor suppressor genes [66], chronic inflammation [67] and etc.
Especially, oncogenes induced replication stress and associated DNA damage results in genomic instability, which alter the gene regulation. When the altered gene regulation activates the genes associated with metastasis then the normal cancer cells acquire the metastatic potential. Apart from genomic instability induced gene expression, oncogenes over expression alone can induce gene expression changes. This is possible because most of the oncogenes are also transcription factors or they regulate transcription indirectly. Further therapy should be based a combination of chemotherapeutic agents along with inhibitors of oncogene.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References


