Cancer and Associated Recent Findings on Metastasis

Eswari Beeram*

Department of Chemical Sciences, Sree Vidyanikethan Degree College, A. P, India-517102

Abstract: *Background:* Cancer is one of the disease with up rised constant mutations and difficult to cure due to metastatic tumors and cell- cell communications between the tumors and the cell through stromal microenvironment and cell signaling.

Discussion: Integrin α11β1 acts as a stromal receptor and remodifies collagen through communication between the stroma and collagen related genes contributing majorly to metastasis in NSCLC. Extracellular matrix protein-1, Osteopontin and Osteonectin are the secreted proteins which are differentially expressed based on the metastatic phenotype. Thrombospondin-1 is responsible for migratory property of the wild cells by promoting intravasation and hyperplasia in many cancers like prostate cancer. Hemopexin and Ferritin light chain (FTL) are the proteins majorly expressed in cancer cell cytoplasm and stromal fibroblasts and contribute to metastasis through secretions mediated by extracellular vesicles.

Conclusion: Other proteins like TWIST, BRCA-1, BRCA-2, cell adhesion proteins, MMPs, Tetraspanin can contribute to metastasis in cancer through promoting either cell migration, Extravasation, Hyperplasia or Angiogenesis facilitating the secondary tumor formation in distant organs.

Keywords: Non small cell lung carcinoma, Hyperplasia, Intravasation, Epithelial mesenchymal transition.

INTRODUCTION

Cancer is one of the major contributors of human deaths worldwide and major reason for poor prognosis of cancer is metastasis and development of secondary tumors. Mutations in proteins involved in cell signaling and genome integrity servesas key contributors of disease development in humans. Integrin α11β1 is one of the structural integral protein and responsible for metastasis in Non small cell lung carcinoma and loss of mutation in Integrin α11β1 is associated with reduced tumor growth and growth factors. Osteonectin (SPARCor BM40) is one of the matrisomalprotein involved in wound repair and healing of Extracellular matrix in bone. Osteonectin is responsible for metastatic potential of many tumors through depleted Type IV collagen and increased leukocyte infiltration. Thrombospondin is responsible for hyperplasia and intravasation of tumors by the rupture of basement membrane and causes metastasis. Epithelial Ovarian Cancer is one of the identified cancers with poor prognosis due to difficulties in diagnosis due to overall spread throughout the body. Fuse binding protein (FBP-1) is one of the cell proliferation protein associated with Epithelial Ovarian cancer and FBP-1 does not affect the apoptosis and cell death. TSP-1 is identified as anti angiogenic factor but high level of expression is seen in metastasized tumors compared to primary tumors in osteosarcoma. Wilm's tumor associated protein (WTAP) regulates RNA splicing mechanisms and its role in alternative splicing is under study now. WTAP is involved in regulating major steps involved in metastasis like cell proliferation, Migration, cell adhesion and morphogenesis.

Matrisomal Proteins and Involvement in Cancer

Integrin α11β1 acts as stromal cell receptor of fibroblasts and acts as one of the major contributor of lung metastasis in Non small cell lung carcinoma (NSCLC). α11 is responsible for collagen cross linking and reorganization in NSCLC and also maintains collagen stiffness. 342 genes are mainly down regulated in NSCLC with $\alpha11^{-/-}$ and majorly include collagen related genes and lysyloxidase like- 1 genes (LOX-1), but LOX-1 is down regulated in other types of carcinomas and protein – protein interactions between α11 and collagen regulated genes and Smooth muscle actin (α-SMA) is may be responsible for metastasis in NSCLC.

$\alpha11^{-/-}$ is associated with reduced tumor growth and the growth factors that are majorly regulated by α11 include latent transforming growth factor beta binding protein 3 (LTBP3) and latent transforming growth factor beta binding protein 4 (LTBP4) in association with transforming growth factor beta 1 (TGFB1), transforming growth factor beta 2 (TGFB2) (TGFβ signaling); WNT1 inducible signaling pathway protein 2 (WISP2), insulin-like growth factor binding protein 2 (IGFBP2), insulin-like growth factor binding protein 4

*Address correspondence to this author at the Department of chemical sciences, Sree Vidyanikethan degree college, A. P, India-517102;
E-mail: eswari.b@vidyanikethan.edu; eswaribeeram@gmail.com

E-ISSN: 2309-6179/22

(IGFBP4)in association with insulin-like growth factor 1 (IGF1) and insulin-like growth factor 2 (IGF2) (IGF signaling); syndecan 4 (SDC4) association with fibroblast growth factor 2 (FGF2) (FGF signaling); semaphorin 3 F (Sema3f) and glypican1 (Gpc1) with vascular endothelial growth factor A (VEGFA) (VEGF signaling); fibulin 1 (FBLN1) and latent transforming growth factor beta binding protein 3 (Ltbp3) with heparin-binding EGF-like growth factor (HBEGF) (signaling via heparan sulfate proteoglycans and EGFR) [1].

α11β1 also interacts with the matrisome proteins of ECM like Glycoproteins, collagens, proteoglycans, ECM secretory and regulated proteins. Expression of α11β1 in stromal cells is majorly responsible for the metastasis of the primary tumors to bone, kidney and brain in NSCLC. Metastasis of tumor cells is a major incurable task and breast cancer metastasis is associated with aggressive lung metastasis reduce the treatment methods in humans. Proteome analysis in metastasized tumors serves to extend the treatment methods available for physicians.RNavab *et al*., developed monoclonal cell lines from the breast carcinoma cell line MDA-MB-435. Major techniques for proteome profiling include chromatofocusing followed by RP- HPLC and separated fractions are subjected to profiling by Mass spectrometry (PMF, ESI- TOF and MALDI TOF) [2].

Secreted proteins like Extracellular matrix protein - 1, Osteopontin and Osteonectin are differentially expressed in metastatic phenotype. Osteopontin is upregulated in majority of cancers and its role in metastasis has studied in MDA-MB-435 and its role in M4A4 cell metastasis is under trials now. Osteopontin is a phosphorylatedsecretory glycoprotein found in all tissues and tissue matrix of mineralized tissues. Osteopontin is a cytokine, cell attachment protein and acts as a signaling molecule for integrins and CD44. Potential role of osteopontin include gene expression, signaling through various growth factors and modulating the proteases responsible for tumor invasion, progression and metastasis.

Osteonectin (SPARCor BM40) is a matricellular protein found in bone matrix. It interacts with the extracellular matrix and involved in wound healing and repair of extracellular matrix. Osteonectin expression is associated with metastatic potential in tumors and is responsible for metastasis through depleted type IV collagen deposition that promotes leukocyte infiltration. Osteonectinupregulation is majorly seen in M4A4 cells compared to MDA-MB-435.

Extracellular Matrix protein-1 (ECM-1) is highly expressed in M4A4 metastatic phenotype than NM2C2 non metastatic phenotype. ECM-1 is a bone matrix protein expressed in basement membrane of Extra Cellular Matrix and promotes angiogenesis in tissues. ECM-1 upregulation is related with either basement disruption or duplication and is responsible for metastasis in many of the cancers like breast carcinoma. ECM-1 higher expression has been detected in 76% of metastatic phenotype and 33% of non metastatic phenotype.

Proteins expressed in non metastatic phenotype include Annexin – I and matrix metalloproteases. Lipocortin or annexin – I is a calcium binding protein of phospholipids and possess anti inflammatory activity and inhibitory activity over phospholipase A2. Lipocortin release is majorly regulated by calcium levels in the cells. Lipocortinupregulation is unclear with most of the metastatic cancers but recently its upregulation in metastatic lung carcinoma was reported. Matrix metallo proteases are involved in most of the metastatic phenotypes and its role in tissue and basement membrane remodeling acts as one of the major factor for tumor invasion and progression in most of the metastatic tumors. MMPs digest majorly Type I, II and III collagens and polymorphism in MMPs promoter is responsible for tumor development in healthy tissues.

Other proteins include cytoskeletal proteins like Cytokeratin, HSP71 and Galectin. These are cytosolic proteins and reaches the extracellular space through exocytosis, phagocytosis and where as Galectin is released through membrane budding of the cells. Thrombospondin-1 is a protein with prometastatic and antimetastatic properties. Endogenous TSP-1 promotes necrosis in tumor cells by inhibiting angiogenesis and mutation of TSP-1 in healthy cells isthe factor that drive them towards metastasis. Thrombospondin regulates the angiogenic switch in tumor cells through macrophage infiltration in healthy and neu- c breast cancer cells. TSP-1 null mice show increased tumor progression and fewer blood vessels thatare larger in size compared to healthy tissues. TSP-1 majorly inhibits tumor growth by activating TGF-β signaling.

Thrombospondin-1 and its Role in Metastasis

Thrombospondin -1 promotes intravasation of tumors in wild type cells and ruptured basement membrane may be reason for intravasation and hyperplasia of tumors. TSP-1 affects the overall tissue

architecture by influencing stromal cell microenvironment and behavior of individual tumors and responsible for migratory property of the wild type cells. ABT-510 is one of the antiangiogenic agent under clinical trials now. It is designated as TSR-2 and derived from sequence GVTIRIR present in second type I repeat of TSP- 1. Recombinant version of TSR-2 was derived by addition of RFK sequence found at N-terminal end of TSP-1 to GVTIRIR repeat. Both TSR-2 and recombinant TSR-2 are tested and found to contain more antitumorigenic potential compared to TSP-1.

Antiangiogenic property of ABT-510 is mediated by CD36 and Arginine 442 is identified as key aminoacid required for anti angiogenic activity of ABT-510. ABT-510 majorly promotes antiangiogenic activity at earliest stage and TGF-β activation at later stage and responsible for optimal tumor progression and metastasis. K.O. Yeeetal., for the first time reported that TSP-1 activates TGF- β and treatment using TSR-2 and recombinant TSR-2 requires 90 days of treatment for optimal suppression of tumor growth.

TSP-1 as mentioned previously an anti angiogenic agent and show higher level of expression in metastasized tumor cells than primary osteosarcoma cells and associated with Enneking stage of osteosarcoma [3]. TSP- 1 mRNA levels are higher in grade III tumors compared to grade IIA and IIB and associated with distant tissue metastasis. TSP- 1 expression is regulated by various cytokines like VEGF, EGF, FBGF and TGF-β1. TGF-β1 found to increase the expression of TSP-1 in osteosarcoma cells. TSP-1 is expressed in time dependent manner and TGF-β signaling is required for metastasis of primary tumors. TSP-1 role in cell proliferation and apoptosis was studied in few of the osteosarcoma cell lines and western blot analysis proved TSP-1 has no role in cell proliferation and apoptosis but required for wound healing in normal tissues.

TSP-1 markedly increases the expression of MMP-2, MMP-9 and Fibronectin- 1 and promotes cell invasion and migration and responsible for metastases of osteocarcoma cells. TSP-1expression was shown to be regulated by p38MAPK, ERK and FAK proteins and recent studies of K.O. Yee *et al.,* on TSP-1 known to promote cell migration and invasion by regulating the FAK signaling and finally MMP-2, MMP-9 and FN-1 expression. TSP-1 *in vivo* targeting in primary tumors does not affect the tumor progression but inhibited the metastasis to distant organs. Previous study on TSP-1 role on metastasis and its affect on the metastasis to distant organs in cancers including breast cancer, melanoma, Thyroid cancer and prostate cancer known to be prevented by knock down of the protein TSP-1.

Positive loop between TSP-1 and TGF-β may exist in osteosarcoma and is responsible for the more aggressive types of osteosarcomas than in TSP-/- tumors. TSP-1 down regulation in p53 deficient tumors may cause tumor progression and death of the mice. TSP-1 expression is majorly increased by stabilising TSP-1 mRNA by p38MAP kinase pathway and positive loop on TGF-β signaling. Silencing of TSP-1and inhibition of FAK signaling resulted in inhibition of metastasis to lung tissues inosteosarcoma and various cancers like breast cancer, prostate cancer, Thyroid cancer, oral cancer without causing affect on growth of primary tumors.

Modulation of MMPs by microRNAs is an important mechanisms regulating MMPs activity [4, 5]. TSP-2 majorly regulates MMP- 2 expression and cell proliferation through interacting with CD36 and integrin αvβ3.Expression of MMP-2 by TSP-2 is MAPK dependent and inhibition of MAPK signaling pathway abolishes the expression levels of MMP-2. TSP-2 levels are usually upregulated in prostate- bone metastatic cancer and levels of TSP- 2 and MMP-2 are positively correlated in expression. TSP-2 is expressed in high amounts in metastatic tumors compared to the localized one. TSP- 2 regulates MMP-2 followed by cell migration, invasion and cancer progression in prostate cancer.

MMP-2 is one of the promigratory protease associated with cell migration and invasion. TSP-2 increase will rise the MMP-2 level and cell migration and invasion by TSP-2 is concentration dependent. Several membranous receptors like CD36, integrin α4β1, and integrinαVβ3 known to mediate the physiological roles in many cancers. Cell migration in prostate cancer is mediated greatly by CD36 and partially through integrin αVβ3. Regulation of CD36 and integrin αVβ3 by TSP-2 is similar to regulation of MMP-2 by TSP-2.

MAPK signaling pathway is a key regulatory pathway involved in cell migration, invasion and tumorigenesis and its down stream regulators p38, ERK, and JNK are majorly phosphorylated on expression of TSP-2 following increase in MMP-2 levels finally regulating cell migration, invasion and tumorigenesis. miR-376c is majorly down regulated by expression of TSP-2 in concentration dependent manner and phosphorylation of p38, ERK, and JNK

regulates miR-376c up on stimulation with TSP- 2 expression. miR-376c levels are highly sensitive to phosphorylated p38, ERK, and JNK and some other independent pathway regulated by TSP-2 maybe responsible for regulating the levels of micro RNA.

Knock down studies of TSP- 2 shown to completely abolish the cell migration and invasion but it does not affect the cell proliferation. MMP- 2 expression is upregulated in many of the bone metastasis and MMP-2 is involved in osteolytic bone migration and finally promoting cell migration in prostate cancer leading to metastasis to bone. TSP-2 expression show positive correlation with MMP-2 and is negatively correlated with miR-376c expression in prostate cancer. TSP-2 is majorly responsible for metastasis in prostate cancer and inhibition of TSP-2 can improve the prognosis and can prevent the metastasis of tumors to bone.

Ovarian Cancers and Key Proteins Involved

Epithelial ovarian cancer is difficult to identify because of its diagnosis possible only after extensive spread all over the body limiting the treatment. Fuse binding protein is a far upstream element increased in different carcinomas like hepatic cellular carcinoma and identified as a oncoprotein. FBP-1 increase has been observed in epithelial ovarian cancer also and understanding the mechanism of cancer development and steps involved in metastasis is required for the better prognosis of EOC.

FBP-1 expression was studied in EOC by using Ki-67 a cell proliferation marker in normal cells, adenoma and carcinoma cells. Highest expression was observed in EOC compared to adenoma and normal cells. FBP-1 knock down in EOC cells does not affect the apoptosis but majorly affects the cell proliferation by preventing the cells from G1/S/G2 transition and it affects cell proliferation by interacting with cell cycle proteins like Cyclin D1/E, c- Myc, p21 and p27 [6].

FBP-1 promotes metastasis through increasing the cell migration by activating the MMP-2. Matrix metalloproteases are the key enzymes involved in digesting the extra cellular matrix and facilitating migration to distant sites in the body. FBP-1 knock down studies can help in to studying the mechanism of tumor development in EOC. Trans well migration studies and wound healing studies on FBP-1 showed positive correlation between the levels of FBP-1 and cell migration and proliferation.

Ovarian cancers can be caused due to mutations in BRCA-1 and BRCA-2 and regulators of MAP kinase pathway and in majority of cases somatic aberrations may be the reason for the cancer development. Previous studies on FBP-1 regulation proved to increase the sensitivity of the cancer cells to carboplatin. Thrombospondin-1 (TSP-1) is identified as a key protein identified in lung metastatic tumor microenviroment and is responsible for metastasis to lungs from primary tumors of osteosarcoma. Microenvironment surrounding the tumor tissue plays a major role in progression and metastasis of primary tumors and thrombospondin levels are found to be higher in metastasized tumors compared to primary tumors. Treatment of lung metastases through recombinant protein is one of the treatments currently available for osteosarcoma. Study on the protein role in cell proliferation, migration and invasion and signaling can improve the therapeutic treatments available in cancer biology.

Role of Axillary Node Metastasis in Breast Cancer

Axillary node metastasis is responsible for unfavorable prognosis in most of the breast cancers and proteome mapping between node positive and negative breast cancers and Primary breast tumors and as well as metastasized breast tumors has identified many key proteins involved in regulation of cell survival and proliferation. Of the 678 proteins 23 proteins showed differential expression between node positive (Tn+) and node negative tumors (Tn-) and proteome analysis can be done with node positive breast tumors and metastasized lymph node tumors also.

Nineteen proteins were upregulated and three proteins are down regulated in Tn+ than Tn^-. Fourteen proteins were upregulated and 23 were down regulated in metastasized lymph nodes compared to primary breast tumors. Four proteins were majorly upregulated including annexin 5, carbonic anhydrase I, peroxiredoxin 6 and proteasome α2 subunit [6]. Eleven proteins were differentially expressed between Lymph node and Tn+ cells and includes 90-kDa heat shock protein, chain A apo-human serum transferrin, heat shock 70 kDa protein 5, chain A α1-antitrypsin, prolyl 4-hydroxylase β subunit precursor, protein disulfide isomerase, β-tubulin, enolase 1, lactate dehydrogenase B, triosephosphateisomerase 1 and macrophage migration inhibitory factor. β- tubulin is differentially expressed both in normal and ALN tumors with high intensity in metastasized tumors [7].

Metastasis associated proteins MTA1 and MTA2 are associated with the process of metastasis due to their interactions with specific protein factors. MTA1 is associated with Histone deacetylase activity where as MTA2 a NuRD ATP dependent chromatin remodeling complex with histone deacetylase activity and localized in the nucleus. Both of the proteins repress transcriptional activity and unlike MTA2, MTA1 does not interact with the universal transcriptional regulators like YY1 or immunophilin FKBP25. Similarly MTA3 is the other metastasis related protein found inside cells but it does not act as transcriptional repressor and show diffused localization inside the cells.

MTA1 and MTA2 proteins are homologus with the N-terminal sequence and divergent in case of C-terminal sequence and MTA1 consists of GATA zinc finger domain, a bipartite nuclear localization signal embedded in a myb DNA-binding domain, and an Src homology 3 binding domain. MTA2 lacks the c-terminal myb DNA- binding domain and is not able to bind to the DNA. The additional function of myb DNA binding domain and relation to tumorigenesis is not clear up to now. In contrast to MTA2 c-terminal domain N- terminal domain is homologus and consist of BAH (bromo-adjacent homology) domain, followed by an ELM2 (Egl-27 and MTA1 homology 2) domain (InterPro, European Bioinformatics Institute).

BAH domain is found in most of the transcriptional regulators including DNA cytosine-5 methyltransferase and the Orc1 (origin recognition complex 1) protein. BAH domain is required for protein – protein interactions and whereas ELM2 domain is a C. elegans homologue of MTA1, Egl27 and MTA1. Many unidentified proteins of C. elegans, A. thaliana and Drosophila melanogaster consists of this ELM2 domain and function of this domain in these proteins are not clear. CoREST, a specific co-repressor for REST/NRSF (RE1 silencing transcription factor/neural restrictive factor) is required for neuronal regulation of gene expression also consists of ELM2 domain.

CoREST also shares another domainnamely SANT (SWI3/ADA2/NCoR/TFIIIB) domain with MTA1 and MTA2. SANT domain has found in other transcriptional regulators like SWI3, yeast SWI/SNF transcriptional activation complex; ADA2 of the ADA activation complex; andNCoR and SMRT are two co-repressors mediating inducible repression by steroid hormone receptors. SANT domain present in juxtaposition and leucine zipper in MTA1 and MTA2 with HDAC1 and HDAC2 confirm the transcriptional activity function of these proteins. Recently identified protein MTA3 with highest homology to MTA1 and MTA2 and might perform distinct function or overlapping functions with MTA1 and MTA2.

MTA1 and MTA2 can repress the transcriptional activity on promoter with Gal4 binding sites where as truncated MTA1 and MTA3 were unable to repress the transcription due to loss of C-terminal domain. C-terminal domain of MTA proteins can acts as transcriptional regulators and required for repression activity of the protein. HDAC1/2 and RbAp46/48 form complex with each other and MBD-3 and Mi-2 are found to be associated with MTA2 and HDAC1/2 and MTA1 and MTA2 showed distinct functions even though they share the common domains HDAC1/2, RbAp46/48 and MBD-3.

Role of Wilm's Tumor Associated Protein (WTAP) and its Isoforms in Cancer

Wilm's Tumor associated protein (WTAP) is a core component of N6- methyl Adenosine (m6A) - methyl transferase complex along with VIRMA, CBLL1, ZC3H13 (KIAA0853), RBM15/15B, and METTL3/14 generates m6A which plays a key role in RNA metabolism. WTAP is a protein and it majorly regulates the splicing mechanisms of RNA and its role in Alternative splicing is a key event under research now. It majorly regulates Alternative splicing in introns rich in GC content and it forms GC Quadruplex formation at splice sites and show inhibitory mechanism on AS at these sites. WTAP is shown to be involved in key events of metastasis like cell cycle, cell migration, cell adhesion and morphogenesis.

Knock down expression and Gene ontology experiments on WTAP and other core components VIRMA, ZC3H13 and CBLL1 showed differential expression of genes 400,900, 1090, 554 [8). Ontology studies of differentially expressed genes detected WTAP as the regulator of cell cycle, cell adhesion, morphogenesis and cell migration. Cell cycle and chemokine secretion is regulated by one of the core component VIRMA, cell migration and cell metabolic activity by ZC3H13, cell adhesion, cell cycle and cell migration by CBLL1.

Differential expression isoforms 1518, 1298, 2086, and 1255 are upregulated by knock down of WTAP whereas isoforms 1293, 1042, 1688, and 983are down regulated by WTAP knock down studies. Five splicing events like alternative 5' site, alternative 3' site, alternate exon skipping/inclusion, microexons and

intron retention are found to be majorly regulated by WTAP and its complex. Knock down studies of WTAP shown to regulate majorly cassette exon skipping/inclusion rather than both exon skipping/inclusion and intron retention of RNA. Knock down of WTAP and ZC3H13 showed high percent of splicing, than compared with VIRMA and CBLL1 along with Exon skipping and intron retention. Similar events are seen with WTAP interacting proteins BCALF1/THRAP3, RBM15/RBM15B, METTL3 and METTL14 also and indicates inhibitory role of these proteins and WTAP.

TWIST and its Role in EMT

TWIST a master regulator of embryo morphogenesis majorly promotes EMT by promoting expression of mesenchymal markers and reduce or induce loss of expression of E- Cadherin a cell adhesion molecule. Different cell lines are characterized from the primary tumors of mammary glands to study the individual steps of metastasis. 4T1 can able to induce metastasis to distant sites resulting in visible metastatic nodules in lung tissue compared to remaining three. Gene expression studies and metastatic ability of primary tumors are analysed to know the contribution of host tissue for the distant site metastasis. Host stromal environment has proved to contribute minimally for the metastasis of primary tumors to lung tissue in > 95% of injected tumor cells.

Gene Expression profile of injected primary tumors include genes that majorly promote intravasation and extravasation and 4T1 show specific gene expression pattern than remaining cell lines and responsible for formation of visible metastatic nodes in lung tissue. In identified genes CXCR3 was reported to be involved in intravasation and generates chemotactic response and invasion in to the tissues. MMP9 an another gene responsible for tumor invasion and metastasis. Many genes similar to CXCR3 are known to involved in breast metastasis and melanomas and found to be upregulated in certain cancers.

TWIST was identified as one of the key regulator protein among the highly upregulated invasion proteins in tumor cells. TWIST expression was not seen in any of the 67NR cells or the immortalized human epithelial cells and high expression was foundto be associated with 4T1 cells. Normally TWIST is responsible for mesoderm formation during embryo morphogenesis and involved in cell migration and proper localization of cells and required for cranial neural tube morphogenesis. Across different phyla TWIST is responsible for cell movement and tissue reorganization during different steps of morphogenesis and its expression is derepressed in most of the mammary tumors and induces EMT and responsible for metastasis and metastatic potential of primary tumors in different organs like lymph nodes, liver and lungs.

Gene targeting of TWIST through SiRNAs resulted in decreased expression of TWIST mRNA and TWIST protein but TWIST does not play a role in tumor proliferation and establishment of primary tumors in tissues but it reduces the metastatic potential of the primary tumors to lungs and the number of visible metastatic nodesin murine models. TWIST majorly regulates the metastasis and it does not affect the micrometastases of nodes to visible tumors. Continuous expression of TWIST is required for the metastasis program to form the visible metastatic tumors in other tissues.

TWIST is required for the invasion and colonization of the mammary cells in lung tissue but it does not support the survival of the anchorage independent colony forming cells in the blood circulation. It does not affect the size of the tumor but is required for the intravasation step in metastatic program [9]. Previous studies of transcription factor TWIST and its role in Drosophila morphogenesis, Atgastrulation TWIST is induced and allow ventral furrow cells to migrate and form mesodermal layer. During this process the cells undergo EMT and increased cell motility which might be responsible for carcinogenesis by TWIST inducing the EMT program and cell motility in cancer cells.

External expression of TWIST in normal cells leads to change in morphological appearance from cobble stone to spindle shaped and loss of cell to cell adhesion contacts and expression of epithelial cell membrane markers like E-cadherin, α-catenin, β-catenin, and deltacatenin and expression of fibroblast markers like fibronectin, vimentin, smooth-muscle actin, and N-cadherin and are positively correlated with TWIST expression and EMT. Similar events are seen in epithelial cells converting them to invasion and tumor inducing phenotypes.

Loss of E- Cadherin is one of the key event in EMT and E- cadherin expression is majorly regulated by silencing the three E- boxes in the promoter of the gene. TWIST repress the E-cadherin mainly by targeting at the promoter level and restoring E-cadherin levels in TWIST expressing EMT cells cannot restore the normal phenotype because of the other

important targets of TWIST like vimentin and fibronectin. TWIST expression was highly elevated in more than 70% of the lobular carcinomas whereas it was about 32% incase of invasive ductalcarcinoma and 30% in mixed ductal and lobular carcinomas, considered as most invasive types of breast tumors.

Invasive lobular carcinoma cells histologically associated with diffuse infiltrative growth, dyshesive single layer of cells where as ductal carcinoma is associated irregular mammary tubules and solid sheet of carcinoma cells. One of the major difference identified between ductal and lobular carcinomas is E-cadherin expression. Lobular carcinomas show complete loss of E- cadherin where as 50% of ductal carcinomas show reduced expression of E- cadherin. TWIST and E- Cadherin expression are inversely regulated in lobular carcinomas a condition responsible for pathogenecity in lobular carcinomas.

Treatment of breast tumors with various chemotherapeutic agents is limited due to undesired side effects and multidrug resistance imposed by the tumor cells. However study of proteome content in plasma membranes of harmone responsive, ErbB2 over expressing and triple negative breast tumors can provide a new sight by targeting the over expressing proteins which are responsible for cell proliferation, cell invasion and cell division through monoclonal antibodies. Cell lines can provide the valid data than tumor tissue because of heterogenicity. Plasma membrane proteins and PM associated proteins which are responsible for proliferation and metastasis can be targeted by studying the proteome through mass spectrophotometry.

Some of the proteins are expressed in unique manner and remaining showed differential expression and showed good correlation among spectra. DHRS2, KRT17, EPHB4, ICAM1, ANPEP, and KIT are the proteins analysed by MS and in these DHRS2, KRT17, and KIT are the uniquely expressed proteins and where as EPHB4, ICAM1, and ANPEP are variably expressed proteins [9,10].

Deregulation of Tyrosine Kinase Receptors can Cause Cancer

Tyrosine kinases are the receptor proteins which are responsible for signal transduction in normal cells and these proteins are mostly elevated in ErbB2 over expressing cell lines. where as TNBC cells show varied expression of tyrosine kinases receptors which accounts for major signaling processes in TNBC cells during metastasis and EMT. Expression of AXL along with Tyrosine kinase receptors on TNBC PM render them insensitive to the tyrosine kinase inhibitors and responsible for disappointing results during treatment. KIT is one of the cytokine receptor expressed on hematopoietic stem cells and various cancer cells and is uniquely found in primary tumor derived cell lines. KIT expression in TNBC varies mostly from 30-90% and higher representation of Ephrin receptor EPHA2 in MDA-MB-231 cells, ErbB3 in DT22 cells, and PTK7 in DT28 cells indicates the various signaling pathways that operate in breast cancer cells to induce growth and cancer progression [10].

Insulin receptor exists in two isoforms, Isoform B is involved in glucose metabolism and isoformA in concert with IGFR1 is involved in cell proliferation. In certain cancer cells interchange between isoform B to isoform A is responsible for tumor progression and it is one of the most important therapeutic target used for treatment of cancer now. One of the primary tumor cell line DT22 shown to express high spectral id for IGFR1 and isoform A compared to other cell lines. When studying the known targets of therapy against cancers most of the therapies are directed against PM based tyrosine kinases and many of these tyrosine kinases are described in many cancers like leukaemia, gastrointestinal stromal cancers and non small cell lung cancer. Many of the similar targets shared by various cancers indicate the need of classifying them based on molecular signatures instead of tissue of origin which can increase the number of therapeutic targets available in BC.

Deregulation of Cell Adhesion Molecules can Promote EMT

Cell adhesion molecules maintain contact between the neighbouring cells and extracellular matrix and involved in signaling transduction and finally cell behavior. So, most of the cancers involve perturbing the cell adhesion process leading to changes in cell proliferation, invasion and cell migration. MS spectra showed high spectral ids for cadherins and catenins, integrins, immunoglobulin superfamily cell adhesion molecules (IgSF-CAMs), two tetraspanins and CD44 markers involved in cell adhesion.

Changes in cadherin expression have been detected in various BC and other certain cancers and change in CDH1 epithelial cadherin to CDH2 neuronal cadherin has been detected in DT22 and DT28 cells is a classic example of cells undergoing EMT. Unique expression of cadherin 19 by DT22 and protocadherin

FAT2 by MCF-10A cells is uncertain and less details is known about these proteins and their role in cancer.

Protein phosphorylation normally regulates various biological functions of the cell. Comparision of phosphorylated proteome and its role in invasion and metastasis may increase the therapeutic targets of pancreatic cancer. Among the upregulated proteome levels,proteome of ErbB and neurotrophin signaling pathways arethe major ones where as p53 and apoptotic signaling pathway proteome levels are majorly downregulated in cancer cells. Among the protein – protein interaction network AKT1 is the majorly upregulated protein in the network and found to have maximum interactions at the proteome level.

Phosphorylated proteome levels of highly and weakly invasive cancer cells are compared by using phosphoantibodies, showed marked upregulation in CaMK1-α protein levels compared to smad 1. Four of the proteins AKT1, Chk2, p53 and P70S6K are differentially expressed in highly invasive cancer cells [11]. Smad1, BID, ACTC1, Smad2, GRK1, AKT1, Cytokeratin 8, c-Raf, Smad2 and PDGF R alpha are top ten down regulated proteins in highly invasive cancer cells compared to weakly invasive cancer cells [11].

FOS, IRS-1 and RAF1 levels are highly upregulated and required for invasion and migration of highly invasive cancer cells and inhibition of these proteins reduced the migration and invasive properties of the cancer cells. Study of highly invasive cells proteome showed remarkable changes in amino acid phosphorylation and also post translational modifications in most of the proteins. Proteins found to be not involved in protein- protein network occupy peripheral portions of the network and CAMK1-α, PIM-1and MKK7/MAP2K7 were no longer included in an up-regulated network, and where as MAPKAPK2, cytokeratin 8, GATA1, GRK1, ACC1, PDGFRA and Smad1 in a down-regulated network when the confidence score was >0.9 analysed by KEGG pathways by X. Tanetal.,(2016).

Among the upregulated ones, proteins involved in metabolism like insulin signaling pathway and cell cycle, proteins involved in immunity like T cell receptor signaling pathway and proteins involved in development like ErbB signaling pathway are majorly upregulated in highly invasive cancers compared to weakly invasive cancer cells. Among PPI network AKT1, CTNNB1, FOS, NFkB-p65, SYK, TP53 and MYC are highly upregulated and BRCA1, LCK, AKT1, and TP53 are mostly downregulated where as AKT1 and Tp53 are the top ten proteins in PPI network known to be downregulated in pancreatic cancers.

Role of Stromal Microenvironment in Promotion of Cancer

Pancreatic ductal adenocarcinoma is one of the most invasive type of cancers followed by lymph node metastasis. Tumor microenvironment plays an important role in overall prognostic rate in these patients. Identification and screening for tumor micro environment proteins involved in metastasis of lymph node can help in targeting the tumor cells and can help in improving the therapeutic prognosis associated with the cancer. Hemopexin is one of the protein found in tumor microenvironment and found to be involved in lymph node metastasis, lymph invasion, venous invasion and increased lymph node ratio [12].

Four proteins including anterior gradient 3, protein disulfide isomerase family member (AGR3); α-defensin 3 (DEF3); myosin heavy chain 14 (MYH14); and abhydrolase domain containing 14B (ABHD14B) were over expressed in the LN− group. Five proteins, hemopexin, ferritin light chain (FTL), α-tropomyosin (TPM1), cysteine- and glycine-rich protein 1 (CSRP1), and plectin were over expressed in the LN+ group. In nine candidate proteins identified in stroma, TPM1 and MYH14 were not identified in any of the tumor stroma but remaining proteins are expressed at varying level.

Hemopexin and FTL are the proteins which are expressed in cancer cell cytoplasm and stromal fibroblasts. They showed focal expression in macrophages, lymphocytes but not in vascular endothelium. Hemopexin and FTL are strongly expressed in LN+ group than LN- group and FTL showed colocalisation with hemopexin [12]. Hemopexin shows positive correlation with clinical pathological parameters associated with lymph metastasis but FTL is not found to be associated with these parameters.

Based on tumor invasion grade classification by Japanese society follows degrees of venous invasion, lymphatic invasion and nerve invasion based on which the tumor is classified in to four groups. Venous invasion and lymphatic invasion were found in hemopexin positive group where as nerve invasion is also found to be severe but not statistically significant. Hemopexin is responsible for invasion of pancreatic ductal carcinoma and lymph metastasis. Based on the wound healing and cell migration studies hemopexin is associated with increasing wound repair capability and

invasion along with cell migration in pancreatic carcinoma.

Extra cellular vesicles are major means of intercellular communication in both physiological and pathological processes and identification of proteins in vesicles and their molecular signatures in diseases like cancer can serve the function of biomarkers and diagnostic factors in physiology and medicine. Recent work by E.M. Guerreiroetal., (2020) Identified some unique proteins in Oral squamous cell carcinoma (OSCC), pancreatic ductal adenocarcinoma (PDAC) and melanoma brain metastasis cell lines with 14%, 10% and 24% of uniqueness in each EVs. Melanoma derived proteins show distinctive properties than compared to OSCC and PDAC. EGFR in OSCC, Muc5AC in PDAC, and FN1 in melanoma vesicles are examples of proteins associated with particular cancer and responsible for cell adhesion, cellular biogenesis and cell motility in these cells.

EV derived from OSCC and PDAC showed positive expression of immune marker CD9 where as melanoma EV showed very low expression of CD9. Proteome analysis showed similarity of 25% where as 7-10% is shared commonly by two of the of EVs and 14%,10% and 24% of proteome was unique and found to present in OSCC, PDAC and melanoma. Proteins in vesicles can be searched with vesiclepedia database. CD9, HSPA8, and ENO1 are the vesicle markers used for validation of data and CD63 is one of the marker uniquely found in melanoma EVs.

Among the top 20 proteins identified EGFR, ITGB4, NT5E, MYO1C, ATP1A1, and CTNNA1 are the enriched proteins found in OSCC and Mucins like (MUC5AC, MUC5B, MUC16, and MUC2), MVP, COL7A1, PGK1, PLEC, and EEF2 are identified in PDAC and HMCN1, HSP8A, APOE, LAMA1, HSP90AB1, CSPG4, PYGB, MYH10, and LAMB1 are found with melanoma brain metastasis. All vesicles shared some proteins commonly like HSPG2, GAPDH, CLTC, ACTG1,PKM, AGRN, and PTGFRN [13].

EV proteins associated with OSCC and PDAC showed hallmarks of cancer like cell adhesion, proliferation and migration. Most of the genes associated with OSCC and PDAC involves genes of integrin family such as ITGA2, ITGA3, ITGA6, ITGA5, ITGB1, and ITGB4. Melanoma EVs contain proteins related to cellular proliferation and cell adhesion proteins with poor expression. ITGB1, ITGA3, and ITGA2 are the genes of integrin family commonly shared by EVs of OSCC, PDAC and melanoma. Molecular signatures of proteins in EVs of melanoma was distinct than other two, in both type and amount of proteins present in them.

EVs proteins in OSCC is found to be involved in various biological processes like biological adhesion, cell motility, interspecies interaction between organisms, cellular localization, cellular component organization, response to wounding, cellular component biogenesis, and interaction with host. Proteins with similar functions are found in PDAC but with low enrinchment scores. Proteins of Evs from melanoma is found with enriched biological processes like interspecies interaction between organisms, biological adhesion, cellular component biogenesis, RNA metabolic process and protein localization, cellular component organization, cellular response to chemical stimulus, organic acid metabolic process, and extra cellular structure organization.

Tetraspanin role in Prostate Cancer

Cell migration is majorly responsible for invasion and metastasis in prostate cancer and Tetraspanin 1 is one of the androgen regulated protein responsible for mortality and morbidity in prostate cancer. Upregulation of TSPAN 1 has observed in both normal and bengin tumor prostate cells and upregulation can lead to metastasis of tumor cells [14]. TSPAN 1 is responsible for cell migration and it upregulates expression of various mesenchymal markers like SLUG and ARF6 and TSPAN1 is the Androgen driven upregulated protein that maintains survival and motility of the prostate cancer cells.

TSPAN 1 is upregulated after 9 hours of androgen exposure and TSPAN1 follows similar dynamics with Androgen receptor directly regulated gene KLK3 [14]. TSPAN1 upregulation is seen under physiological androgen levels and start sequence of TSPAN1 overlaps with the AR binding site and regulated by androgens synthesis and androgen binding to AR. Induction of TSPAN1 expression although observed after 9 hours oftime period, detectable protein levels are observed only after 48hrs of AR activation. TSPAN1 levels are upregulated in prostate carcinoma relative to begnign prostate carcinoma and primary prostate tissue compared to normal one.

According to data from UNIPROT, TSPAN1 is a transmembrane protein and its localization is both cytoplasmic and membrane bound. Microarray analysis technique can be used to know the distribution and

upregulation of TSPAN1 in prostate cancer patients *in vivo*. Radical prostatectomy showed no correlation with the patients survival and relapse free survival with levels of TSPAN1. A striking change in expression pattern of TSPAN1 was observed in metastatic prostate tumors than in the primary prostate tumors. TSPAN1 is not associated with cell proliferation and adhesion but it targets cell migration in prostate tumors.

TSPAN 1 mainly targets genes responsible for EMT (Epithelial Mesenchymal Transition) and cell motility, migration. Transcriptional repressor SLUG and small GTP- binding protein ARF 6 are also detectable on upregulation of TSPAN1. SLUG expression is seen in advanced stage of pancreatic carcinoma and associated with EMT. SLUG expression is also observed in cancers with neuroendocrine phenotype. TSPAN 1 is required for cell viability in prostate cancer cells and down regulation of TSPAN 1 is associated with decrease in phosphorylated ERK1/2 protein resulting in down regulation of Ras- ERK1/2 signaling.

TSPAN 1 is involved in invasion of cervical cancer cells, cell proliferation and migration of colon cells *in vitro*, promote invasion and survival of skin carcinoma cells and play a role in survival, proliferation and carcinogenesis of pancreatic cancer cells. Regulation of TSPAN1 by micro RNAs promotes cell proliferation and invasion in colorectal cells and cell migration in non small cell lung carcinoma. ARF-6 is mainly responsible for cell motility in prostate cancer cells and modulated by TSPAN1. A study in cervical cancer excluded the role of TSPAN 1 in PI3K signaling and FAK signaling.

TSPAN CD151 is elevated in prostate cancer and responsible for spontaneous tumor induction in mouse models and TSPAN 8 is also upregulated in prostate cancers and involved in cell invasion. Tetraspanin CD9 is down regulated in prostate cancer and is a suppressor of metastasis in mouse models. TSPAN 1 is one of the top 30 listed genes in prostate cancer screened using mass spectrometry and used as a marker of aggressiveness in prostate carcinoma.

Immunotherapy and Role in Breast Cancer Prognosis

Breast cancer is one of the cancer that ranks first in complexity with genomic alterations, protein expression deregulation, signaling pathway alterations, hormone disruption, ethnicity and environmental determinants are involved. Targeting the proteins in BC is one of the trending topic in drug design. RPS27, SUPT4H1, CLPSL2, POLR2K, RPL38, AKT3, CDK3, RPS20, RASL11A and UBTD1 are the top ranked immunotherapy proteins; S100A9, DDA1, TXN, PRNP, RPS27, S100A14, S100A7, MAPK1, AGR3 and NDUFA13 are top ranked metastasis directing proteins and S100A9, TXN, RPS27L, RPS27, RPS27A, RPL38, MRPL54, PPAN, RPS20 and CSRP1 are top ranked RNA binding proteins in BC [15].

Identification of immunotherapy proteins in cancer oncology is astriking feature due to long term responses associated with the advanced stages of cancer and metastasis. Among the 10 identified proteins RPS27, SUPT4H1, CLPSL2, POLR2K, RPL38, AKT3, CDK3, RPS20, RASL11A, and UNTD1; RPS27 is identified as a tumor associated antigen in BC [15]. Detecting the genes that undergo genomic alterations in BC can provide a valuable insight in therapeutics. POLR2K, ASH2L, MED30, NSL1, RPRD2, CDC73, EIF3E, SRP9, HNRNPU and SNRPE are the genes associated with frequent genomic alterations identified and MYC, OBSCN, ASH2L and BRD4 are with highest number of cDNA mutations, mRNA alterations and protein alterations, respectively.

Metastasis is one of the difficult aspects to treat and is often accompanied by the tumor resistance there by hindering the treatment. Metastasis driven genomic alterations rely on mutations andgene signature patterns different from primary tumors. In the top most identified proteins S100A9, DDA1, TXN, PRNP, RPS27, S100A14, S100A7, MAPK1, AGR3 and NDUFA13 are the metastasis driven proteins;S100A9 is identified to present in estrogen receptor negative and progesterone receptor negative breast cancers and causes risein inflammatory cytokines and associated with impaired survival rate in BC cases.

YWHAZ, PTK2, SETDB1, EBAG9, MTBP, NUCKS1, ATAD2, PIK3CA, HSF1 and TP53 are top 10 metastatic proteins with frequent genomic alterations and MYC, PIK3CA, SETDB1 and BRD4 carried the highest number of cDNA mutations, mRNA alterations and protein alterations, respectively. RNA biology is of the critical factor to be considered due to pleiotropic change seen in gene expression in many of the cancers. RBPs play a important role in RNA biology such as translation, splicing, stability, degradation of mRNA, nucleocytoplasmic transport, capping, and polyadenylation. S100A9, TXN, RPS27L, RPS27, RPS27A, RPL38, MRPL54, PPAN, RPS20 and CSRP1 are the top 10 identified RBPs and TXN is over

expressed in BC and known to contribute to tumor grading, being a key element in redox homeostasis. EIF3H, KMT2C, DCAF13 and EEF2 carried the highest number of cDNA mutations, mRNA alterations and protein alterations, respectively.

Analysis of coexpression of millions of cells is now possible through flow cytometry by using single cell population isolated from complex tissue. Flow cytometry can be used to know the heterogenecity of lung tissue in metastatizedmelanoma tumors through commercially available plate based antibody screening panels to achieve simultaneous analysis of surface markers on millions of single cells isolated from complex tissue. CD31, CD41, Clec12a, and CD200R3 are the markers used to differentiate basophils from eosinophil in FcεR1+ cells. CD55, CD95, CD279 (PD-1), and CX3CR1 are the markers that are heterogeneously expressed in CD8T cells in the blood identified by the infinity flow computational pipeline [16].

CD45 showed co expression with markers identified on CD8T cells and infinite flow is able to separate recently described B1 (glycosylated CD43+ IgD− CD21/35− CD272−) and B2 (glycosylated CD43− IgD+ CD21/35+ CD272+) subset of B cells. Naïve cells (CD44− CD38− CD62L+ CD45RB+) and previously activated cells (CD44+ CD38+ CD62L− CD45RB−)can also be separated by infinite flow of CD4T cells in flow cytometry (16). Macrophages ingest the tumor cells in lungs during metastatic seeding of lungs and it is possible to identify the macrophages with ingested tumor cells by using high expressing CD64 and low expressing CD26 markers and with high expression of MHC class II.

MHC class I molecules are expressed in almost all nucleated cells of body. Function of these molecules include processing and presentation of Ag to cytotoxic T cells and abnormal expression of this molecule on cancer cells help in immune protection against cancer. But modulation of MHC- I expression on PM of cancer cells can make them undetected by immune system in certain cancer cell lines like DT28, SKBR-3, and MCF-7 cells rather than in MCF-10 A cells. Interestingly some of the cancer cells usually upregulate the expression of MHC-I molecules and this uncertainity is due to difference in expression of MHC-I mRNA and protein on PM of cancer cells. It is difficult to detect the MHC-I on PM of cancer cells by immunostaining due to destruction of cell surface epitopes of the protein. DT22 and MDA-MB-231 cells show high expression of MHC-I proteins rather than MCF-10A cells however using MHC I as a target for treatment is unclear up to now.

REFERENCES

[1] R. Navab *et al*., Integrin α11β1 regulates cancer stromal stiffness and promotestumorigenicity and metastasis in non-small cell lung cancer. Oncogene; 2016; 35: 1899-1908. https://doi.org/10.1038/onc.2015.254

[2] P. Kreunin. Identification of metastasis-associated proteins in a human tumor metastasis model using the mass-mapping technique. Proteomics; 2004; 4(9): 2754-2765. https://doi.org/10.1002/pmic.200300767

[3] K.O. Yee *et al*., The effect of thrombospondin-1 on breast cancer metastasis. Breast Cancer Res Treat.; 2009; 114(1): 85-96. https://doi.org/10.1007/s10549-008-9992-6

[4] Chidambaram K, Willoughby DA, *et al*., MicroRNA regulates MMP-9 expression. J Ocular Biology, Diseases and Bioinformatics, 2010; 3: 41-52. https://doi.org/10.1007/s12177-010-9054-2

[5] Chen *et al*. Thrombospondin-2 promotes prostate cancer bone metastasis by theup-regulation of matrix metalloproteinase-2through down-regulating miR-376cexpression. Journal of Hematology & Oncology; 2017; 10(33): 1-17. https://doi.org/10.1186/s13045-017-0390-6

[6] Xiong *et al*., FBP1 promotes ovarian cancer development through the acceleration of cell cycle transition and metastasis. oncology letters 2018; 16: 1682-1688. https://doi.org/10.3892/ol.2018.8872

[7] Ya-Li Yao and Wen-Ming Yang. The Metastasis-associated Proteins 1 and 2 Form Distinct ProteinComplexes with Histone Deacetylase Activity. The journal of biological chemistry; 2003; 278(43): 42560-42568. https://doi.org/10.1074/jbc.M302955200

[8] Keiko Horiuchi, Kawamura T, Hamakubo T. Wilms' Tumor 1-Associating Protein Complex regulates alternative splicing and polyadenylation at potential G-quadruplex-forming splice sitesequences, Journal of Biological Chemistry 2021. https://doi.org/10.1016/j.jbc.2021.101248

[9] Yang *et al*., Twist, a Master Regulator of Morphogenesis, Plays an Essential Role in Tumor Metastasis. Cell, 2004; 117: 927-939. https://doi.org/10.1016/j.cell.2004.06.006

[10] Y.S. Ziegler *et al*., Plasma Membrane Proteomics of Human Breast Cancer Cell Lines Identifies Potential Targets for Breast Cancer Diagnosis and Treatment. PLOS ONE; V 9(7): e102341. https://doi.org/10.1371/journal.pone.0102341

[11] X. Tan *et al*., Phosphoproteome Analysis of Invasion and Metastasis-Related Factors in Pancreatic Cancer Cells. PLOS ONE; 2016. https://doi.org/10.1371/journal.pone.0152280

[12] Suzuki *et al*., Stromal expression of hemopexin isassociated with lymph-node metastasis inpancreatic ductaladenocarcinoma. PLOS ONE; 2020. https://doi.org/10.1371/journal.pone.0235904

[13] E. M. Guerreiro *et al*., Cancer cell line-specific protein profiles in extracellular vesicles identified by proteomics. PLOS ONE; 2020. https://doi.org/10.1371/journal.pone.0238591

[14] J. Munkley *et al*., The cancer-associated cellmigration protein TSPAN1 isunder control of androgens and itsupregulation increases prostatecancer cell migration. Scientific Reports; 2017; 7: 5249. https://doi.org/10.1038/s41598-017-05489-5

[15] A. López-Cortés *et al*., Prediction of breast cancer proteins involved in immunotherapy, metastasis, and RNA-binding

usingmolecular descriptors and artificial neural networks; Scientific Reports; 2020; 10: 8515. https://doi.org/10.1038/s41598-020-65584-y

[16] E. Becht *et al*., High-throughput single-cell quantification of hundreds of proteins using conventional flow cytometry and machine learning Sci. Adv.; 2021; 7: eabg0505 https://doi.org/10.1126/sciadv.abg0505

Received on 15-09-2022 Accepted on 19-10-2022 Published on 12-11-2022

DOI: https://doi.org/10.12974/2309-6179.2022.07.04