Role of Carcinoma Associated Fibroblasts in Anoikis Resistance in Oral Squamous Cell Carcinoma – need of the hour

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INTRODUCTION

Anoikis is a protective mechanism against invasion and metastasis [1]. Anoikis resistance (AR) is a major beneficial quality governs by cancer cells as it bears intrinsic characteristics for metastasis.

Tumor microenvironment (TM) plays a crucial role in tumor progression, invasion and metastasis. Array of research found out that CAFs which showed myo-fibroblasts like features, are responsible for cancer invasion and metastasis [2,3]. CAFs helps the cancer cells to travel to distant site through metastasis by inhibiting anoikis of tumor cells [4].

AR occurs in OSCC through array of mediators [5]. It was found that anoikis resistant OSCC cell lines displayed a unique karyotypic and genotypic fingerprint that differs from anoikis sensitive OSCC cells [5]. It has been proved that CAFs causes inhibition of anoikis in breast cancer cells [4]. CAFs help the cancer cells to evade anoikis. CAFs are important cell in tumor stroma responsible for invasion, metastasis and has an important effect on AR in tumor cells [4,6].

Effect of CAF on AR in OSCC is a field of research. So for the first time, we would like to focus the light on interrelationship of CAFs and molecular pathway in anoikis resistance phenomenon responsible for metastasis. Many a time CAFs showed chemoresistant property for cancer cells in OSCC patients. So revealing the role of CAFs in anoikis phenomenon will help in providing a new roadway for therapeutic purpose.

Different Molecular Pathways for Anoikis Resistance via Cafs

Epithelial-mesenchymal transition (EMT) is a key process for metastasis and...
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exhibits an essential characteristic for AR. It is associated with increase in expression of proteins like N cadherins, smooth muscle actin etc [7]. It was found that N cadherins were able to induced AR in tumor cells [8].

CAFs play the role in AR through various mechanisms for the cancer cells.

CAFs facilitate invasion via EMT. Expression of Transforming growth factor (TGF) β via CAFs induces EMT which results in downregulation of E cadherins and up regulation of N cadherins, which leads to AR [1,6,9]. Also, TGF β expressed via CAFs is responsible for loss of E cadherin which prevents anoikis as E-cadherin-interacting protein, ankyrin-G, arbitrates anoikis regulatory signals [10].

TGF-β and FGF-2 assist with each other and regulate EMT during cancer progression. During EMT, TGF-β induces isoform switching of fibroblast growth factor (FGF) receptors. It causes the cells to become sensitive to FGF-2 and formed “activated fibroblast” which encourages the formation of complexes between ZEB1 (Zinc finger E-box-binding homeobox 1) and CtBP1 (C-terminal-binding protein 1). These complexes phosphorylated through the FGF-2- activated MEK-ERK pathway. Moreover, activated ERK pathway triggers factor ZEB1 via up regulation of FRA1 (Fos-related antigen 1) which is responsible for EMT by inhibiting anoikis of malignant cells. Concurrently, cancer cells invade and metastasize at distant site in cooperation with activated fibroblasts [10].

CAFs exhibits two vital processes for EMT which helps in overcoming anoikis. Firstly, they secrete Matrix metalloproteinase (MMP) 2 and 9 which were found to cleave the E cadherins. Secondly, CAFs aid in release of reactive oxygen species (ROS) via Rac1b/cyclooxygenase-2 pathways which helps the cancer cells to overcome anoikis for EMT [11].

Researchers found out that CAFs had stabilizing effect on antiapoptotic proteins which were responsible for anoikis. Study done on breast cancer cells revealed the critical role of CAFs in blocking anoikis through Insulin like Growth factor receptor (IGFR). It was unrevealed that CAFs stabilizes antiapoptotic protein Mcl-1 via IGFR and inhibits anoikis of cancer cells through paracrine manner [4].

CAF enhances EMT in cancer cells and increase their metastatic potential via regulation of stem-cell traits. Subsequently, EMT causes activation of pathways by constitutively activating specific pro-survival signals such as PI3K/AKT pathway signals which leads to AR [12]. Also, in prostate cancer, TGF β signaling in fibroblasts results in promotion of cancer cells. TGF-β ligand BMP2 induces invasion via STAT3, ERK1/2 and AKT activation by stimulating anoikis resistance [12,13]. TGF β expressing by CAFs has an effect on the MAPK/ERK and PI3K/AKT signaling pathways and these pathways are responsible for the increase in resistance to anoikis in cancer cells [14].

Research has proved that “anoikis resistant CAFs” inhibits anoikis of cancer cells by paracrine signaling and carry them to distant site for metastasis [15,16].

A study done by Horowitz et al. proved that TGF β prevents anoikis of myofibroblasts (MF). TGF β 1 induces rapid activation of P13/AKT pathway. Activation of P13/AKT pathway is mediated by p38 MAPK-dependent production of growth factors which acts in an autocrine/paracrine mechanism. Thus, initiation of P13/AKT pathway results in induction of “anoikis resistant phenotype” to MF at the time of EMT [15].

A detailed study on anoikis and MF proved that TGF β regulates SMAD-mediated N-cadherin expression for EMT. TGF β has a coordinated and independent role in activation of FAK (Focal adhesion kinase) and AKT kinase pathways through the stimulation of SMAD3 and p38 MAPK factors for endowing “anoikis resistant phenotype” to MF [16].
Kim et al. revealed that invasive breast cancer cells granted an AR phenotype on MF by activating laminin-332 up regulation and neoexpressing integrin β 4. Two mechanisms were put forward; indirect interaction, direct interaction. In indirect interaction, factors released from cancer cells increases the expression of laminin-332 by MF. Laminin-332 binds to integrin α3β1 present on MF and "switch on" the autocrine cell survival signal through AKT pathway. Direct interaction involved the direct contact between invasive breast cancer cells and MF results in induction of neoexpression of integrin β4. MF cell-survival signals were mediated by Rac1 activation and AKT phosphorylation [17].

**INTERPRETATION**

1. In epithelial cancer, AR is essential to set free from epithelium.
2. In OSCC, lymph node metastasis is because of mobility of cancer cells by modulating actin cytoskeleton and forming invadopodia.
3. From various studies, it is evident that CAFs expressing TGF-β controls the motility of cancer cells by regulating pathways like AKT, SMAD, MAPK involved in AR etc.
4. CAFs indirectly and directly control the expression of array of molecules for AR.

Interrelationship between CAFs and AR has a definitive role in metastasis. Till now CAFs has been explored in OSCC in array of roles, however its role for AR has still to be explored and conceptualize. AR has a major impact on prognosis of the patient as it is a prerequisite for metastasis. A direct role of CAFs on anoikis is yet to unreveal in OSCC which bears a distinct effect on cancer cell survival during invasion. A better understanding of molecular mechanism involved in AR via CAFs to overcome metastasis would help in the formation of novel anticancer approach to attained better prognosis.

**REFERENCES**

1. Kim YN, Koo KH, Sung JY, Yun UJ, Kim H. Anoikis Resistance: An Essential Prerequisite for Tumor Metastasis. International Journal of Cell Biology. 2012; 11. [Ref.: https://goo.gl/WdRz6A]
2. Martinez-Outschoorn UE, Balliet RM, Rivadeneira DB, Chiavarina B, Pavlides S, et al. Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution A new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. Cell Cycle. 2010; 9: 3256-3276. [Ref.: https://goo.gl/NmYRJU]
3. Taguchi A, Kawana K, Tomio K, Yamashita A, Isobe Y, et al. Matrix Metalloproteinase (MMP)-9 in Cancer-Associated Fibroblasts (CAFs) Is Suppressed by Omega-3 Polysaturated Fatty Acids in vitro and in vivo. PLoS One. 2014; 9: e89605 [Ref.: https://goo.gl/W6iy5l]
4. Weigel KJ, Jakimenko A, Conti BA, Chapman SE, Kaliney WJ, et al. CAF-Secreted IGFBPs Regulate Breast Cancer Cell Anoikis. Mol Cancer Res. 2014; 12: 855-866. [Ref.: https://goo.gl/sBjtCq]
5. Bunek J, Kamarajan P, Kapila YL. Anoikis Mediators in Oral Squamous Cell Carcinoma. Oral Dis. 2011; 17: 355-361. [Ref.: https://goo.gl/LDcTTN]
6. Yu Y, Xiao CH, Tan LD, Wang QS, Li XQ, et al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-β signaling. Br J Cancer. 2014; 110: 724-732. [Ref.: https://goo.gl/VII6YD]
7. Nagafuchi A, Shirayoshi Y, Okazaki K, Yasuda K, Takeiichi M. Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. Nature. 1987; 329: 341-343. [Ref.: https://goo.gl/RB7PmQ]
8. Li G, Satyamoorthy K, Herlyn M. N-cadherin-mediated intercellular interactions promote survival and migration of melanoma cells. Cancer Research. 2001; 61: 3819-3825. [Ref.: https://goo.gl/SKBDwo]
9. Pavlides S, Vera I, Gandara R, Sneddon S, Pestell RG, et al. Warburg Meets Autophagy: Cancer-Associated Fibroblasts Accelerate Tumor Growth and Metastasis via Oxidative Stress, Mitophagy, and Aerobic Glycolysis. Antioxid. Redox Signal. 2012; 16: 1264-1284. [Ref.: https://goo.gl/1aqEaa]
10. Shirakihara T, Horiguchi K, Miyazawa K, Ehata S, Shibata T, et al. TGF-β regulates isoform switching
of FGF receptors and epithelial–mesenchymal transition. The EMBO J. 2011; 30: 783-795. Ref.: https://goo.gl/MQW8uB

11. Giannoni E, Bianchini F, Calorini L, Chiarugi P. Cancer associated fibroblasts exploit reactive oxygen species through a proinflammatory signature leading to epithelial mesenchymal transition and stemness. Antioxid Redox Signal. 2011; 14: 2361-2371. Ref.: https://goo.gl/SbJcZp

12. Paoli P, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta. 2013; 1833: 3481-3498. Ref.: https://goo.gl/nwMakG

13. Smith BN, Bhowmick NA. Role of EMT in Metastasis and Therapy Resistance. J Clin Med. 2016; 5: 17. Ref.: https://goo.gl/eaZ96n

14. Muñoz NM, Baek JY, Grady WM. TGF-β has paradoxical and context dependent effects on proliferation and anoikis in human colorectal cancer cell lines. Growth Factors. 2008; 26: 254-262. Ref.: https://goo.gl/iYuwMJ

15. Horowitz JC, Lee DY, Waghray M, Keshamouni VG, Thomas PE, et al. Activation of the Pro-survival Phosphatidylinositol 3-Kinase/AKT Pathway by Transforming Growth Factor1 in mesenchymal cells is mediated by p38 MAPK-dependent induction of an autocrine growth factor. The journal of biological chemistry. 2004; 279: 1359-1367. Ref.: https://goo.gl/m20lI8

16. Diamond ME, Sun L, Ottaviano AJ, Joseph MJ, Munshi HG. Differential growth factor regulation of N-cadherin expression and motility in normal and malignant oral epithelium. J Cell Sci. 2008; 121: 2197-2207. Ref.: https://goo.gl/uhYHs6

17. Kim BG, Gao MQ, Choi YP, Kang S, Park HR, et al. Invasive breast cancer induces laminin-332 upregulation and integrin β4 neoexpression in myofibroblasts to confer an anoikis-resistant phenotype during tissue remodeling. Breast Cancer Research. 2012; 14: R88. Ref.: https://goo.gl/K9O1Jo