Oral Squamous Cell Carcinoma and Anoikis: A Brief Review on Recent Advances

Anjali Ganjre¹, Gargi S Sarode², Sachin C Sarode³, Shankargouda Patil⁴

Contributors:
¹Senior Lecturer, Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India; ²Associate Professor, Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India; ³Professor, Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India; ⁴Associate Professor, Department of Maxillofacial and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Saudi Arabia.

Correspondence:
Dr. A. Ganjre, Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Sant-Tukaram Nagar, Pimpri, Pune - 411 018, Maharashtra, India.
Phone: +91-9922491465. Email: drsachinsarode@gmail.com

How to cite the article:

Abstract:
Anoikis resistance is a crucial process responsible for metastasis of cancer cells in oral squamous cell carcinoma (OSCC). Array of molecules is responsible for modulation of anoikis which directly or indirectly results in anoikis resistance. They alter the specific molecules like hepatocyte growth factor, caveolin, tetramolybdenum which play an important role in anoikis. Moreover, they modify different pathways such as Akt, mitogen-activated protein kinase, Wnt/catenin to overcome metastasis. It will be beneficial to review anoikis associated recent advancements in the English medical literature so that it will help in impeding novel therapeutic approaches to overcome metastasis and thus improving the prognosis of the OSCC patients.

Key Words: Anoikis, invasion, metastasis, oral cancer, oral squamous cell carcinoma, prognosis

Introduction
Oral squamous cell carcinoma (OSCC) is the most common cancer of oral cavity, which accounts for more than 90% of oral malignancies.¹ In India, the age standardized incidence rate of oral cancer is 12.6 per 100,000 population showing noteworthy sharp increase in the incidence rate of oral/pharyngeal cancers.² The most common cause of mortality in OSCC patients is metastasis, which is responsible for more than 90% of oral cancer-related deaths. 40% of patients show lymph node metastasis which is the major determinant in the prognosis of OSCC patients.¹

At cellular level, metastasis leads to the detachment of cellular islands from the epithelium and navigate to the distant sites via lymphatic route. To achieve the distant sites, the cancer cell has to prevent anchorage-dependent cell death known as anoikis. It is indirectly a safeguard mechanism against the metastasis. To overcome these death signals and for the sake of survival, malignant cells have to prevent anoikis and acquire resistance to anoikis attributes.³ Various cell lines experiments have suggested that cancer cells from primary OSCC showed more resistance to anoikis as compared to normal oral keratinocytes. Thus, cells showed aggressive behavior, resistance to radiation, chemotherapy, and exhibit metastatic phenotype.⁴ Therefore, anoikis resistance bears major setback in the prognosis of the patients as therapeutic approaches targeted against it seem to be unraveling and insufficient. Understanding the pathogenesis of metastasis through anoikis resistance is the need of the hour and has to be unrevealed. It will be helpful in providing new biomarkers and modes of therapy against metastasis. Hence, it is our sincere effort to review the array of biomarkers studied in anoikis resistance, which will be helpful in tailoring novel therapeutic options in the treatment of OSCC (Table 1).

Anoikis
In physiologic conditions when a cell gets detached from the epithelium, the cell cycle is arrested and the cell undergoes a rapid caspase-mediated cell death, known as anoikis. However, cells bearing cell junctions cause cell-specific activation of integrins and their downstream signaling mediators, which help to promote cell survival through interactions with cytoplasmic kinases, small G-proteins, and scaffolding proteins. Integrin association activates focal adhesion kinase (FAK) and causes autophosphorylation of FAK and other cellular proteins. Autophosphorylation of FAK at Y397 offers a binding site for SH2 domain-containing proteins such as Src family kinases and PI3K subunit p85. Activation of these signaling pathways is critical for anoikis resistance.⁵

In metastasis, Src activation leads to phosphorylation of FAK on tyrosine 397, which results in recruitment of PI3K in lamellipodia. Subsequently, activated Akt inhibits apoptosis by regulating cell death machinery through Bim. Furthermore, Src-mediated activation of FAK enhances antiapoptotic components like Bel2 associated death promoter by Akt and inhibits caspases, thereby suppressing anoikis.⁶ Gaining anoikis resistance or anchorage-independent survival is a hallmark of oncogenic transformation.
of HGF was responsible for the increased expression of Cox-2. Furthermore, it was found that inhibition of Cox-2 activity is attenuated by HGF-mediated anoikis resistance.8

Swan et al. had demonstrated the phenomenon of anoikis resistance in various cell lines derived from human tissues, that is, oral keratinocytes to invasive OSCC and metastatic carcinoma of tongue squamous cell carcinoma. It was found out that metastatic tumor cells bear more anoikis resistant characteristics as compared to cells of primary tumor cells.9

FAK is a tyrosine kinase molecule responsible for the invasion and metastasis of squamous cell carcinoma. It was revealed that extracellular matrix (ECM) survival signal transduced by FAK suppress p53 mediated apoptosis. Suppressing P53 increases resistance for the anoikis in cancer cells. Co-relationship of FAK signal transduction and p53 will result in reduction of anoikis and increased cell survival for metastasis.10

Estrogen receptor signaling was found to be responsible for the cancer formation, progression, and metastasis in breast cancer. Interestingly, it was studied that estrogen receptor had a role in anoikis resistance. FAK acts via mitogen-activated protein kinase (MAPK) pathway and modulate the cytoskeleton F-actin. Ishida et al. studied the effect of ER in OSCC and found out that ER antagonist inhibited FAK phosphorylation for invasion and metastasis. Tamoxifen acts as an ER antagonist and induces anoikis in OSCC cells with a decrease in p-FAK while ER agonist helps in invasion of cancer cells in OSCC.11

Wnt/beta-catenin signaling pathway plays a crucial role in tumorigenesis, invasion, and metastasis. Yang et al. showed that Wnt/beta-catenin regulate anoikis, which depends on the death receptor signaling pathways. Thus, they are responsible for cell invasion and survival of the cancer cells. It was found that overexpression of Wnt/beta was directly correlated with anoikis resistance in cancer cells.12

AKT/PI3K pathways are found to be helpful for survival, invasion, and metastasis of cancer cells in OSCC. Signal transduction through AKT/PI3K pathway is responsible for anchorage-independent survival and inhibition of this pathway. It was found to be responsible for apoptosis of cancer cells. Mandal et al. did the study on AKT specific inhibitor, KP372-1. It was revealed that blocking Akt kinase by selective inhibitor KP372-1 causes apoptosis of oral cancer cells growing in culture. It inhibited phosphorylation and kinase activity of Akt which subsequently causes induction of BAX expression and cleavage of poly (ADP-ribose) polymerase. Thus KP372-1 potentially contributes to the apoptosis and anoikis-inducing effects. Moreover, it was found that KP372 related AKT was revealed to be inhibited other kinases, such as CDK1, CK2, CSK, DNAPK, ERK1, GSK3b, LCK, MEK1, PIM, PKA, protein kinase C, and S6K. Thus, high levels of Akt phosphorylation will be correlated with aggressive behavior of the tumor.13

### Table 1: Anoikis related molecular markers studied in OSCC.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Year</th>
<th>Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeng et al.3</td>
<td>2002</td>
<td>HGF</td>
</tr>
<tr>
<td>Zeng et al.4</td>
<td>2002</td>
<td>HGF</td>
</tr>
<tr>
<td>Swan et al.5</td>
<td>2003</td>
<td>Cell lines</td>
</tr>
<tr>
<td>Zhang et al.6</td>
<td>2004</td>
<td>FAK and p53</td>
</tr>
<tr>
<td>Yang et al.7</td>
<td>2006</td>
<td>Wnt/beta catenin</td>
</tr>
<tr>
<td>Mandal et al.8</td>
<td>2006</td>
<td>Akt inhibitor KP372-1</td>
</tr>
<tr>
<td>Ishida et al.9</td>
<td>2007</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>Timpson et al.10</td>
<td>2007</td>
<td>Cortactin</td>
</tr>
<tr>
<td>Kupferman et al.11</td>
<td>2007</td>
<td>Anoikis resistant gene analysis</td>
</tr>
<tr>
<td>Zhang et al.12</td>
<td>2008</td>
<td>Caveolin</td>
</tr>
<tr>
<td>Neiva et al.13</td>
<td>2009</td>
<td>STAT, ERK, Akt</td>
</tr>
<tr>
<td>Kumar et al.14</td>
<td>2010</td>
<td>Tetramethylrhodamine</td>
</tr>
<tr>
<td>Campos et al.15</td>
<td>2012</td>
<td>Endothelial derived factor (VEGF)</td>
</tr>
<tr>
<td>Kamarajan et al.16</td>
<td>2012</td>
<td>RIP and SIRT3</td>
</tr>
<tr>
<td>Xie et al.17</td>
<td>2013</td>
<td>p53</td>
</tr>
<tr>
<td>Zhang et al.18</td>
<td>2014</td>
<td>EGF</td>
</tr>
<tr>
<td>Arnold et al.19</td>
<td>2014</td>
<td>Rac1</td>
</tr>
<tr>
<td>Savar et al.20</td>
<td>2015</td>
<td>Alpha v, p53</td>
</tr>
<tr>
<td>Dey et al.21</td>
<td>2015</td>
<td>S100A7</td>
</tr>
<tr>
<td>Yadav et al.22</td>
<td>2015</td>
<td>FAK and p53</td>
</tr>
<tr>
<td>Wilting et al.23</td>
<td>2016</td>
<td>miRNA</td>
</tr>
</tbody>
</table>

F: Focal adhesion; HGF: Hepatocyte growth factor; miRNA: MicroRNA; Rac: Rac1; T: Tetramethylrhodamine; FAK: Focal adhesion kinase; HGF: Hepatocyte growth factor; miRNA: MicroRNA; Rac1: Rac1; T: Tetramethylrhodamine; FAK: Focal adhesion kinase; HGF: Hepatocyte growth factor; miRNA: MicroRNA

### Discussion

Here we have strategically discussed the various molecules, which can either suppress or enhance the phenomenon of anoikis. The list of markers and associated studies are shown in Table 1.

Hepatocyte growth factor (HGF) is a cytokine responsible for proliferation, growth, invasion and metastasis via c met oncogene in head and neck squamous cell carcinoma (HNSCC). It was found that HGF strongly suppresses anoikis and provide anoikis resistance to the malignant cell. HGF is responsible for the activation of Akt, extracellular signal-regulated kinase (ERK), nuclear factor kB (NF-kB) pathways which are responsible for the anoikis resistance in malignant cancer cells. Inhibition of ERK and AKT plays a role in suppressing HGF mediated anoikis resistance pathways. Moreover, it was revealed that inhibition of anoikis by HGF was independent of NF-kB pathway because NF-kB-induced anti-apoptotic genes including A1 and cellular FLICE inhibitory protein are not activated by HGF.7

In a study by Zeng et al., it has been showed that ERK pathway stimulated activator protein 1 (AP-1) was critical for HGF dependent anoikis resistant survival of cancer cells. Moreover, it was found that the anti-apoptotic gene cyclooxygenase-2 (Cox-2) was induced by HGF in an AP-1 dependent manner. Anchorage-independent survival of HGF expression was dependent upon ERK. Inhibition of ERK signaling blocks Cox-1 pathway by HGF. Overexpression of Cox-2 was found to be associated with HNSCC cancers, and constitutive activation of HGF was responsible for the increased expression of Cox-2. Furthermore, it was found that inhibition of Cox-2 activity is attenuated by HGF-mediated anoikis resistance.8

Wnt/beta-catenin signaling pathway plays a crucial role in tumorigenesis, invasion, and metastasis. Yang et al. showed that Wnt/beta-catenin regulate anoikis, which depends on the death receptor signaling pathways. Thus, they are responsible for cell invasion and survival of the cancer cells. It was found that overexpression of Wnt/beta was directly correlated with anoikis resistance in cancer cells.12

AKT/PI3K pathways are found to be helpful for survival, invasion, and metastasis of cancer cells in OSCC. Signal transduction through AKT/PI3K pathway is responsible for anchorage-independent survival and inhibition of this pathway. It was found to be responsible for apoptosis of cancer cells. Mandal et al. did the study on AKT specific inhibitor, KP372-1. It was revealed that blocking Akt kinase by selective inhibitor KP372-1 causes apoptosis of oral cancer cells growing in culture. It inhibited phosphorylation and kinase activity of Akt which subsequently causes induction of BAX expression and cleavage of poly (ADP-ribose) polymerase. Thus KP372-1 potentially contributes to the apoptosis and anoikis-inducing effects. Moreover, it was found that KP372 related AKT was revealed to be inhibited other kinases, such as CDK1, CK2, CSK, DNAPK, ERK1, GSK3b, LCK, MEK1, PIM, PKA, protein kinase C, and S6K. Thus, high levels of Akt phosphorylation will be correlated with aggressive behavior of the tumor.13
Cortactin is ubiquitously present Src substrate responsible for protein–protein interaction, which aid in tumor progression. It is encoded by CTNN gene. Gene interacts with actin cytoskeleton present in lamellipodia and helps the cell for invasion. Cortactin plays a direct role in regulating actin reorganization during cell migration, binding and activating the Arp2/3 complex, and stabilizing branched actin networks. It binds preferentially to dynamic actin filaments. In vitro study showed that cortactin-knockdown cells exhibit a lower production of uncapped actin filament barbed ends at the leading edge, which related to a reduced persistence of lamellipodial protrusions. Timpson et al. found out that cortactin increases the expression of HGF by activating ERK and p13 pathway which was responsible for disassembly of adheren junction. Thus, it helps to overcome anoikis and support anchorage-independent survival of malignant cells. Interestingly, differential gene expression profile unrevealed a list of important genes which play a role in anoikis resistance. According to Kupferman et al. anoikis resistant cells exhibits different type of gene expression. They have compared differential gene expression in anoikis resistant cell line and anoikis sensitive cell lines with the help of karyotyping and polymerase chain reaction analysis. When cells get detached from the epithelium, they show differential expression of genes for anoikis resistance. They have revealed that anoikis resistant cell lines express certain gene expression such as S100P, KLK6, and CTNNAL1. They were overexpressed in anoikis resistant cell lines to correlate with aggressive behavior.

Cavolin-1 (Cav-1) is the main constitute of cavolae and is a scaffolding molecule for several signaling molecules like endothelial growth factor (EGF). One school of thought put forward is that the reduced level is related with the formation of cancer while others suggested overexpression is the cause of tumor formation. According to a study done by Zhang et al., overexpression of Cav-1 increases the sensitization of metastatic cells for anoikis via direct interaction with integrin β1 and src. It disrupts integrin/src mediated metastasis and invasion. Thus, overexpression of Cav-1 induces anoikis and inhibits metastasis. Furthermore, it was revealed that significant down-regulation of Cav-1 co-relates with the lymph node metastasis and that Cav-1 expression is inversely associated with N-stage.

Endothelial cells play an active role in tumor progression and metastasis. It was found that signaling pathways initiated by endothelial cells were responsible for activation of signal transducer and activator of transcription 3 (STAT3), AKT, and ERK pathways. In vitro experiment revealed that endothelial cells release interleukin-6 for STAT3 activation, CCLX8 for Akt pathway and EGF for ERK activation. Blockage of specific pathways in endothelial cells has direct effect on inhibition of migration of cancer cells and invasion. It was found that copper-dependent kinases (lysyl oxidase) were involved in metastasis and invasion via FAK. Kumar et al. showed the effect of tetramolybdenum on cancer cells and found out that TM induces anoikis in malignant cells by activating p38 MAPK cell death pathway and by down-regulating X-linked inhibitors of apoptosis (XIAP) survival proteins. It was found that TM chelates copper-dependent kinases and causes anoikis in the detached cell. Moreover, TM promotes cancer cell anoikis by upregulating oxidative stress in cancer cells and by down-regulating superoxide dismutase and XIAP in which copper acts as a co-factor.

Cancer stem cells are the drivers of progression and invasion of cancer. Campos et al. showed the co-relationship of endothelial factor on cancer stem-like cells for anoikis. It was found that endothelial-derived vascular EGF (VEGF) activates p13 Akt pathway which results in head and neck cancer stem cells metastasis. It was revealed that endothelial cells express VEGF and activate Akt/P13 signaling which results in anoikis resistance in CD44 and aldehyde positive cells (cancer stem like cells) as compared to CD44- and aldehyde-cells of HNSCC.

Receptor interacting protein (RIP) helps in inducing anoikis in cancer cells. It shuffles between CD95/Fas death and FAK survival signaling pathways to mediate anoikis in OSCC cancer cells. In anoikis condition, FAK and RIP dissociate which leads to the formation of death inducing signaling complex.

Sirtuins (SIRT1–7), the mammalian homologs of the SIR2 gene in yeast and found to be overexpressed in OSCC. SIRT1-7 mediates anoikis resistance in OSCC and is a negative regulator of RIP. Kamarajan et al. showed that SIRT and RIP expressed oppositely in OSCC in anoikis resistance cells, and these support the cells to escape anoikis and adapt more aggressive behavior phenotype. Multicellular aggregate is an essential prerequisite to escape anoikis process. Moreover, it was found that RIP and SIRT both were critical for regulating reactive oxygen species (ROS). Thus according to a study, RIP and SIRT3 control anoikis resistance via ROS regulation pathways.

P53 signaling pathways regulate anoikis of OSCC cells. It was revealed in “in-vitro” study that single OSCC cell when detached exhibits lower levels of FAK phosphorylation than the multicellular OSCC aggregates. Fibronectin permits cell–cell adhesion and confirms resistance to anoikis. Moreover, it was found that repressing FAK function increases anoikis. It has been proved that FAK promotes anchorage-independent growth and migration of OSCC cells, which is vital for metastatic phenotype. FAK and p53 are interlinked with each other and important for regulating ECM survival signals. ECM survival signals transduced by FAK inhibit p53-mediated apoptosis by preventing upregulation of p53 by death-associated protein kinase. Moreover, suppressing p53 function...
in SCC cells reduced anoikis in SCC cells reinforcing the link between FAK and p53 in for anoikis in SCC cell aggregates. VEGF is responsible for progression and proliferation in OSCC. VEGF increases in low hypoxic area. VEGF secrets EGF and was found that EGF down-regulates E-cadherins resulting in loss of cell-cell junction. EGF induces motility and prevents the cancer cells from anoikis. Endothelial cell initiates signaling enhancing tumor cell proliferation, migration, and anoikis resistance. EGF activates major pathways such as STAT3, ERK, and PI3K/Akt which were responsible for the induction of anoikis resistance in tumor cells.

Rac 1 is a molecule responsible for the increase in sensitivity of cancer cells for anoikis prevention. Arnold et al. conducted the study on IRR HNSCC cells and HNSCC cell lines. It was found that IRR cells showed increased Rac 1 expression. Activation of ErbB signaling was revealed in IRR cells. There was increased expression of total EGFR, ErbB2, and ErbB3 receptors. IRR HNSCC cell lines exhibit amplified expression of Akt/protein kinase B (PKB) and down-regulation of phosphatase and tensin homolog (PTEN). PTE acts as a repressor of the PI3K/Akt/mTOR pathway associated with activated ErbB family receptors. Down-regulation of PTEN leads to activation of the pro-survival PI3K/Akt/mTOR intracellular pathway followed by Rac1 activation resulting in anoikis resistance. Rac 1 can be a crucial regulator of Notch-1, c-myc, E-cadherin and PTEN expression and activity. When cell is detached, it survives due to the activation of above pathways. Inhibition of Rac 1 activity and expression results in interruption of metastasis by restoration of anoikis process in cancer cells.

Moreover, Savar et al. found that inactivation of Akt in OSCC cells, which lack the expression of p53, and av integrin results in anoikis. Loss of p53 and av activates AKT pathway, which prevents the cancer cells from anoikis and allows survival.

Psoriasin belongs to S100 A7 gene family and responsible for inflammatory response in skin. Its increased in expression is related with oncogenesis in various cancers. Altered expression is responsible for poor prognosis. Psoriasin gene is present on epithelial differentiation chromosomal locus and in cancer, the gene is responsible for de-differentiation process. Thus, altered level of psoriasin is results in oncogenesis and invasion. Moreover, psoriasin regulatory factor regulates cell-cell attachment. Dey et al. found upregulation of S100A7 mRNA and proteins in resistant cells. They were responsible for anchorage-independent growth and thus phosphorylation of Akt pathway. Thus, it was directly responsible for anoikis resistance and metastasis of tumor cells. Inhibition of PKB results in inhibition of S100A7 survival pathway.

Tumor associated endothelial cells are present in the malignant tumors and are found to exhibit high Bcl2 expression and directly correlated with the promotion of metastasis in HNSCC. Yadav et al. showed that EC-Bcl2 positive cells express E-selectin via Ras/Raf/MAPK signaling pathway which help in de-attachment of tumor cells. Bounded cells showed significantly higher anoikis resistance, which was mediated by the Src-FAK signaling pathway. Activating circulating endothelial cells were shown to bear high level of Bcl-2 protein for apoptosis. It was found that activated CSC co-migrate with the tumor cells to distant sites. Attached EC-Bcl-2 cells with tumor cells showed higher anoikis resistance via the activation of Src-FAK pathway.

Wilting et al. found out the co-relationship of DNA methylation-mediated silencing of microRNAs (miRNAs) in attaining anchorage-independent survival for migration of cancer cells. They have proved that methylation-mediated silencing of tumor suppressive miRNAs contributes to acquisition of an anchorage-independent phenotype and thus help in achieving anoikis resistance.

Moreover, further studies on various histopathological variants of OSCC especially in acantholytic variant can form an interesting area of research. Acantholytic OSCC is considered as an aggressive but rare type of OSCC characterized by tubular and alveolar patterns as a consequence of the acantholysis with the loss of immunohistochemical expression of E-cadherin, causing loss of cell adhesion in the center of the tumor nests. It would be interesting to explore association of cancer associated microflora, mitochondrial stress, and oral potentially malignant disorders with anoikis resistance in future studies.

Conclusion

OSCC is a major public health concern. In spite of various treatment plans like chemotherapy, radiations and radicular surgical resection implicated in OSCC, there is no significant improvement in the prognosis of the patient. The survival rate of <50% over the 5 years have been observed. Early diagnosis and treatment will improve the mortality rate for OSCC patients as late diagnosis renders the tumor cells for metastasis. According to various researches, anoikis resistance makes OSCC more aggressive, resistance to treatment and more prone to metastasis. For understanding the detailed mechanism of anoikis survival in OSCC, it is noteworthy to review the array of molecules actively participating in the anoikis process. It will not only highlight the novel biomarkers of anoikis but also aid in providing therapeutic targets to improve the prognosis of the patients. It will definitely lay a foundation for more therapeutic lines in future.

References


2015;10(10):e0141602.


