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# 3,3′- DIINDOLYLMETHANE (DIM) AS A NOVEL POTENTIAL CHEMOPREVENTION FOR BARRETT'S ESOPHAGUS TO ESOPHAGEAL ADENOCARCINOMA: A MINI-REVIEW ON SIGNALING PATHWAYS

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**ABSTRACT**
Barret esophagus is one the most important and dreaded consequences of chronic gastroesophageal reflux disease that can significantly increase the likelihood of esophageal adenocarcinoma in these individuals. Despite extensive research in regard to the diagnosis and treatment of said condition, there is no standardized treatment yet. Besides anti-reflux drugs, many substances have been suggested as potential candidate for chemoprevention of adenocarcinoma, but none has been widely accepted. Authors of this paper have proposed 3,3′- Diindolylmethane (DIM) as a potential effective chemoprevention for esophageal adenocarcinoma. DIM is an indole component that is predominantly found in cruciferous vegetables. Authors have reviewed the most important pathways that result in the development of esophageal adenocarcinoma from Barret's esophagus and how DIM can affect them. Some of these pathways are as follows: Growth factors and other cellular stimuli (C-Met, Cyclin complexes and cyclin-dependent kinases), MAPK and PI3K pathway, Shh, Notch, Wnt signaling pathway, the Hippo pathway, aryl hydrocarbon receptor (AhR), Nuclear factor kappa-light-chain-enhancer of activated B, tumor suppressor's pathways (P53, P27 and P21). It seems that DIM can be a promising substance for stopping the progression of barret's to adenocarcinoma.

**KEY WORDS:** 3,3′- Diindolylmethane (DIM), Barrett's esophagus, esophageal adenocarcinoma.

## INTRODUCTION

One of the challenging aspects of modern medicine is the discovery of many conditions that despite their proven effect of disease progression, there is no consensus on their treatment. Many new meta-analyses have challenged previous cancer screening protocols which were once seen as a life saving measure. Maybe the most prominent examples of this trend are the PSA screen. PSA screen is recently found to cause almost no difference in overall survival despite earlier detection of prostatic.[1] This issue is also present in patients who undergo upper gastrointestinal endoscopy and receive a diagnosis of Barret's esophagus. Although anti-reflux therapy is the mainstay of treatment in these patients, it does not stop the progression of Barret's esophagus to adenocarcinoma.[2-6] Lack of cost benefit and effective treatment for this condition has put a shadow of doubt on the importance and necessity of screens.[5-9] Many trials have been tried for a better treatment regimen without any success. One of the newer substances with a promising effect on this issue is DIM. Efficacy and safety of this substance is proven both in vivo and in vitro as both prophylaxis and treatment in many cancers.[11] It seems due to potential effects of DIM it can be a suitable candidate for chemoprevention of Progression of Barrett's esophagus to adenocarcinoma. In this paper, we discussed the effects of DIM on important pathways that lead to adenocarcinoma and the possibilities of using it as an effective drug in our fight against cancer.

### Barrett's esophagus and the challenges we face

Barrett's esophagus is defined as metaplasia of the lining of distal esophagus which replaces at least 1 cm of the stratified squamous epithelium and changes it to the columnar epithelium.[2-5] Barrett's esophagus is the only identifiable premalignant lesion and the most common precursor for esophageal adenocarcinoma. Many studies suggest at least a 6-fold increase in the incidence of this cancer from the 1970s till today. Despite many discoveries and innovations in screening and treatment, the survival rate is still not satisfactory.[2] The impact of Barrett's esophagus can be more readily understood when it is shown that having this condition increases the chances of developing adenocarcinoma by 0.12-0.5% each year and some researchers even claim that in 95% of esophageal adenocarcinomas, the diagnosis of Barrett's has been missed at some point.[3-4]

Barrett's esophagus (with respect to the level of dysplasia) is histophathologicaly classified as negative for dysplasia, indefinite for dysplasia, low grade

dysplasia and high grade dysplasia. This classification is important in patient management and achieving the best result possible. According to American College of Gastroenterology (ACG) guidelines, in patients with low grade dysplasia, high grade dysplasia and intramucosal carcinoma, endoscopic ablative therapy is recommended.[5] Unfortunately, Endoscopic Eradication Therapy in patients without dysplasia isn't deemed cost-effective and is not recommended by experts due to many reasons like low probability of progression of Barrett's to full-blown adenocarcinoma, high number needed to treat (NNT), risk of recurrence, high cost, need for periodic endoscopic surveillance and lack of high-level evidence of this treatment.[6] Currently, these patients are put on a reflux control regiment using Proton pump inhibitors (PPIs) and are followed by upper GI endoscopy every 3-5 years (5-shaheen). Although there is some controversy regarding the use of PPIs that question the chemopreventive effect of these drugs. Furthermore, some studies even suggest that PPIs can have a role in increasing the level of gastrin which in turn leads to more proliferation of epithelial Barrett's cells and can have a carcinogen effect in itself.[7, 8]

Some of the latest studies have suggested a link between use of statins and aspirin as a chemopreventive method in Barrett's Esophagus. But the current state of evidence and lack of adequate randomized controlled trials which doesn't allow researchers to clearly show a cost-benefit result for them, and the relatively high NNT (NNT=389 for statins), is the most cited reason for their lack of routine use in clinical settings as an antineoplastic agent.[5,9] Currently, there is a global effort for finding prophylactic agents for different cancers; one promising candidate is dindolylmethane (DIM).

**How Barrett's Esophagus progresses to adenocarcinoma?**

Studies show that chronic reflux of stomach contents to the esophagus and the stress of this encounter, in time changes the squamous epithelial cells to columnar type cells, this pre-cancerous lesion is what we usually call Barrett's Esophagus. To put it in a nutshell, this change includes two different components: firstly the genetic variation in telomerase, P53, P16, and APC genes, secondly interaction of reflux content with pathways of Wnt/Notch/Hedgehog and homeobox gene Cdx2.[10]

**3,3'-Dindolylmethane (DIM) and its properties**

DIM is an indole component which can be found in abundance in cruciferous vegetables like broccoli and cabbage. Chemical structure of DIM includes an indole (an aromatic heterocyclic organic compound) with a six membered ring that connects to five-membered nitrogen-containing pyrrole ring. Plants rich in DIM have been used medicinally in alternative medicine for a long time to treat various ailments. Nowadays with the development of new lab techniques and through various randomized controlled trials (RCTs) these compounds have shown a lot of promise in treatment and prophylaxis of a wide range of diseases including but not limited to: leiomyoma, HPV infection, RSV virus, atherosclerosis and some cancers. Many communities based and epidemiologic studies as well as in-vitro and in vivo studies have demonstrated the anti-tumoral effects of DIM in many organ systems like breast, colon, liver, cervix, endometrium and lung.[11]

**Signaling pathways and molecular mechanism in Barrett's esophagus and the effect of DIM on them**

Like other types of cancer, the underlying mechanisms which cause the Barrett's esophagus develop into adenocarcinoma is a combination of both genetic and epigenetic factors. Although the full picture is still unknown, we can say with a fair degree of certainty that genetic mutations, problems in gene repair, cellular checkpoints, and disturbances in apoptosis are the main suspects that contribute to this process. By far the most important factor is reflux of stomach contents into the esophagus.[10]

What follows is a short review into the most important processes that are essential to the development of adenocarcinoma from Barrett's esophagus (fig. 1):

- **Growth factors and other cellular stimuli:**

**Epidermal growth factor (EGF):** EGF family like EGF and transforming growth factor-α (TGFα) are very pivotal for the stimulation of growth in epithelial cell proliferation. Studies suggest the level of EGFR protein expression is about 67% in all cases of esophageal adenocarcinomas which is identical to the increase that is observed in Barrett's esophagus. Recently Ohashi et al have shown that EGFR overexpression is an important factor in epithelial to mesenchymal transition (EMT).[12] Also, erbB-2 which is another member of the EGFR family is strongly suspected to be a key player in the late stages of development of esophageal Barret's (high-grade dysplasia) into adenocarcinoma.[13, 14] This has been successfully shown in a study in which targeting erbB-2 in Her2 positive patients with metastatic esophageo-gastric junction cancer was effective in reduction of disease burden.[15]

Other important factors in metastatic esophageal adenocarcinoma are the simultaneous expression of VEGF-C and $VEGFR_3$.[16] So it seems the factors that can inhibit the branching pathways from epidermal growth factor (EGF) family, can also be used as a potential treatment for barret's. Kandala et al have worked on different types of cell line in ovarian cancer and also have shown that DIM can inhibit phosphorylation of mitogen-activated protein kinase (MEK) and extracellular signal-regulated kinase (ERK) which leads to decrease in EGFR activity (this effect is shown both in-vitro and in-vivo studies.[17] Likewise, the inhibitory effect of DIM on EGFR has been supported by many studies in cancer, breast and lung cancers.[18-21]

Several studies have shown that transforming growth factor β (TGFβ) has a critical role in the inhibition of

tumor-forming and differentiation in many epithelial cancers including esophageal adenocarcinoma.[10] Interestingly, Lee et al. have shown that DIM can express nonsteroidal anti-inflammatory drug-activated gene-1 (transforming growth factor β superfamily gene) independent of P53 in breast cancer.[22]

**C-Met**: Another contributing factor in the progression of dysplasia in Barret's and esophageal adenocarcinoma is overexpression of C-Met. In short, it seems that the effect of C-Met on cells of esophageal adenocarcinoma is decreased in expression of E-cadherin which leads to overexpression of β-catenin that can increase the cancerous activities in the cell.[23] The good news is that new studies by Nicastro et al. on the effect of DIM on breast cancer cells have shown that it can decrease the phosphorylation of C-Met at tyrosines 1234 and 1235 that reduces the activity of receptor as an inhibitor in the signaling pathway of C-Met.[24]

- **Kinases transcription factors and other effectors**

**Cyclin complexes and cyclin-dependent kinases:** One of the important mechanisms that contribute heavily to Barret's pathogenesis and its progression to adenocarcinoma is impairment in cellular cycles such as cyclin complexes and cyclin-dependent kinases. This can readily be observed in overexpression of nuclear cyclin D1.[25-27] Also with amplification of 19q12 (the location of the gene for cyclin E), we can find an increase in another group of cyclins called cyclin E.[28-29] So, the control and decrease in activity of cyclins should always be an integral part of a successful strategy for treatment of Barret's esophagus and preventing its progression to adenocarcinoma. This connection is better understood with the knowledge of some gene polymorphisms like G870A polymorphism with reflux disease and their relation to impairments in cyclin complexes and kinase-dependent cyclins which usually result in higher rates of metastasis and lower survival.[30] Studies conducted on DIM on human esophageal squamous cancer cells have clearly shown that DIM can inhibit cyclin activity in vitro very well. Kim et al. showed that DIM had an impressive reduction in cyclin D1, cyclin E2, cyclin-dependent kinase (CDK) 4 and CDK 6 activities. Other important findings in this study were the role of DIM in activation of caspase 9 through the increase in activity of CDK inhibitors p 15 and other apoptosis markers in line with G1-Phase cell cycle arrest.[31]

**MAPK and PI3K pathways**: Another important element in cell proliferation, growth, differentiation and progression to Barret's is the upregulation of MAPK and PI3K pathways.[32] These act as crucial parts of inhibition of tyrosine kinase receptor pathways. In an in-vitro study, Zhu et al. showed that DIM could significantly downregulate MAPK and PI3K in human cervical cancer cell lines.[33] So we can postulate that this antitumoral effect of DIM can also be used against the progression of Barret's to dysplasia.

**Shh, Notch, Wnt signaling pathways**: Other signaling pathways involved in tumor formation and progression of BE to EAC are Shh, Notch, Wnt. These pathways are physiologically part of cellular proliferation and differentiation before birth and in hemostasis of adults.[34-36] This signaling pathway uses the membrane Frizzled (FZD) receptors and low-density lipoprotein receptor-related protein (LRP) to stimulate and aggregate β-Catenin around the nucleus which is a key part of the carcinogenic process of BE (34-36). Dim also impacts the Wnt pathway. Li et al. have shown that it can inhibit phosphorylation of GSK-3β and β-catenin which leads to less activation of Akt/GSK-3β in prostate cancer and regulates this pathway.[19]

**E-Cadherin** is another important factor in tumor invasion that decreasing this protein in cellular membrane led to increasing the chance of progression to EAC.[37, 38] With this in mind, Jin et al. used DIM on colorectal cancer cell lines and saw a significant increase in E-cadherin mRNA level and its product in DLD-1 and HCT116 cells.[39]

**The Hippo pathway** is part of yes-associated protein (YAP) cascade and negatively regulates kinase associated cascades of apoptosis and stops cell growth.[40] DIM strengthens the linkage of RASSF1 to Mst1/2- LATS1-MOBI1 complex and induces hippo pathway and via phosphorylation of YAP results in less cellular proliferation.[41]

**Aryl hydrocarbon receptor (AhR)** is a protective agent in many cancers such as esophageal. AhR activates by specific ligands and formation of ligand-AhR complex that induces cell cycle arrest in G1 phase and apoptosis tumor.[42] Recent studies have shown, in esephegeal cancer, there is a direct correlation between hypermethylation of the AHRR promoter region and tumor dysplasia. This signifies the importance of this connection. Fortunately, Yun et al. have clearly shown that DIM can be used by this mechanism to stop stomach cancer from progression.[43] Interestingly in recent publication Degner et al. discovered a relationship between exposures to AhR ligands like dioxins with induction of COX-2. As is known COX-2 is a key enzyme in prostaglandin E2-mediated transactivation of EGFR and JNK activation that is important in the pathogenesis of many cancers including EAC[44,45] Increase in COX2 also equals more inflammation which indirectly accelerates cancer formation. Many studies clearly show that cox-2 has a link with GERD and BE progression to adenocarcinoma.[46] There is hope that DIM and its derivatives can stop COX-2 overexpression and epigenetically blunt AhR activation and in so doing, stop the progression of BE to adenocarcinoma.[47]

**Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB):** NF-κB is an extremely important complex in BE pathogenesis. Production of this factor is observed in many stresses and in response

to cytokines and environmental effects. Many studies suggest that NF-κB increases in esophageal epithelial cells when they are exposed to bile and the level of reactive oxygen species rises in them. This leads to chronic inflammation and activation of COX-2 and progression to EAC.[48-51] Some studies show a downstream transcription effect from DIM on NF-κB.[52] Clark et al have shown that DIM can inhibit NF-κB in human colorectal cancer cells.[53] This effect has also been shown by Rahman et al. in breast cancer cells.[54]

**Tumor suppressor's pathways**

**P53**: Undoubtedly, one of the most crucial elements in the regulation of cell cycle is P53. Mutations in tumor-suppressing genes especially P53 can impair apoptosis pathways. This particular disturbance is quite common in many types of cancer including esophageal carcinoma and it seems to have a profound effect in the pathophysiology of progression to it.[55] Weng et al. showed that DIM could inhibit oral squamous cell carcinoma cell line by an increase in p53 signaling. It can also affect Ser-15 phosphorylation can induce p53 activation that leads to more apoptosis via Puma and Noxa pathways.[56]

**P27**: Expression of P27 is reduced in many cases of esophageal adenocarcinoma (EAC) (57). DIM activates the caspase 9 that leads to an increase in its expression.[31]

**P21**: Although the expression of P21 increases in dysplastic BE and EAC but P21 is not able to down-regulation of the cell cycle. So, it seems that we would need to increase the P21 activity in order to decrease in cyclin complexes and cyclin-dependent kinase activities.[58] The study of Hong et al. on the effect of DIM on breast cancer cells showed that DIM by the activation of SPL/SP3 of p21 led to amplification of this pathway which causes the cell arrest in G1 phase.[59]

**SUMMARY**

This mini-review show that DIM can be a potential chemoprevention treatment for Barret's via the stimulation or inhibition of main pathways including growth factors and other cellular stimuli (C-Met, cyclin complexes and cyclin-dependent kinases) MAPK and PI3K pathway, Shh, Notch, Wnt signaling pathway, the Hippo pathway, AhR, nuclear factor kappa-light-chain-enhancer of activated B and tumor suppressor's pathways (P53, P27 and P21). Although many in-vitro studies in recent years have shown the efficacy of DIM as a promising treatment but the main hurdles of its usage in clinical practice are the low bioavailability and undermined safety. Also DIM have paradoxically, induced tumor in rare cases.[60] So, it seems DIM although hopeful in chemoprevention of esophageal adenocarcinoma, needs more in-vitro and in-vivo (animal models) research before human trials can begin.

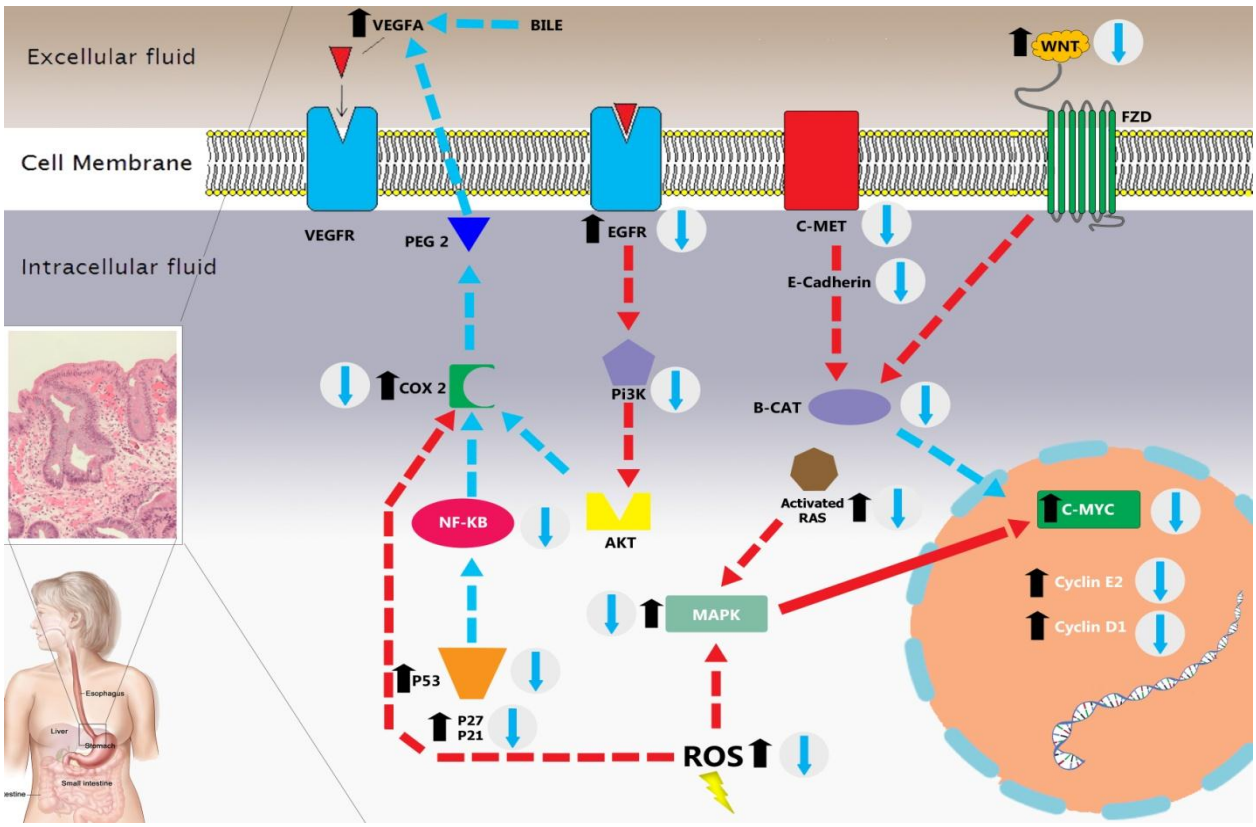


**Fig 1. The effects of DIM on signaling pathways and molecular mechanism of Barrett's esophagus and it's progression to esophageal adenocarcinoma (dotted blue arrow: stimulation of expression, dotted red arrow: increase in the activity, black arrow: increase in the carcinogenicity of the component or molecule, Solid blue arrow: the anticancer effect of DIM on the component).**

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