# PROGNOSTIC SIGNIFICANCE OF DEIODINASE ENZYMES MRNA EXPRESSION IN BREAST CANCER

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**ABSTRACT**

**BACKGROUND**

Iodothyronine deiodinases type 1, 2 and 3 (DIO1, DIO2 and DIO3) are enzymes that activates and deactivate Thyroid Hormones (TH) maintaining their homeostasis at cellular level. Recent studies show a possible role of deiodinases in various cancers. In this manuscript; we used data mining analytical approach and studied the prognostic significance DIO1-3 in Breast Cancer.

**METHODS**

Prognostic values of DIO1-3 were predicted using the "Kaplan- Meier Plotter" (KM plotter) database for direct in silico validation in clinical cases of breast cancer patients. After extracting KM survival plots, Relapse Free Survival (RFS) were compared, and Hazard Ratio (HR) and log-rank p-values were calculated.

**RESULTS**

There was a significant change in RFS of ER-ve breast cancer patients, with high DIO1 and DIO2 expression. Further, ER+ve breast cancer with hormone therapy high expression of DIO3 results in significantly poor RFS.

**CONCLUSIONS**

We found limited potential of prognostic values of DIO1-3 in breast cancer. We conclude that in some subgroups of breast cancer, these findings may be beneficial as a companion diagnostic predicting more accurate breast cancer prognosis.

**KEYWORDS**

Breast Cancer, Deiodinases, Relapse Free Survival, Kaplan Meier, KM Plot

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## BACKGROUND

Thyroid hormones (THs) are key regulators of various physiological process including growth, proliferation, differentiation, cell death, and metabolism. THs are synthesized and secreted from the thyroid gland. Hypothalamic thyrotropin-releasing hormone signals the pituitary gland to produce and secrets thyroid-stimulating hormone which activates TH synthesis and secretion.[1] Thyroid hormones released from gland enters into blood stream and reaches to target tissue to exerts its effect at cellular level.[2]

Deiodination of less active hormone thyroxin to more active form (Triiodothyronine) is a critical process that requires catalytic deiodination performed by deiodinases.[3] T4 is converted to physiologically functional T3 via removal of outer-ring iodine by the type 1 and 2 deiodinases (D1 and D2). The type 3 deiodinase (D3), and under several circumstances D1, can inactivate T3 and T4 by the removal of inner-ring iodine, generating T2 or reverse T3 (rT3).[4] Thyroxin acts as a key hormone that exerts its effects via formation a complex with nuclear thyroid hormone receptor that in turn binds to thyroid hormone receptor elements on specific targeted genes and modulates their levels at transcriptional level.[5] T3 apart from regulating at genomic levels T3 can regulate metabolic activity by directly acting at mitochondrial level termed as its non-genomic effect.[6] Breast cancer is the most common cancer in women worldwide. According to a recent estimation of over 508, 000 women died in 2011 due to breast cancer.[7] TH being critical regulators of cell proliferation, differentiation, apoptosis and metabolism can determine cell fate. Extensive research has supported the existence of the relationship between thyroid hormone and pathophysiology of various cancers.[8] Oncogenesis is effected by deregulation of bio availability of TH at cellular levels that critically determined by the enzymatic activity of deiodinases.[9] Therefore, we perform an analyzed the prognostic role of DIO1-3 in human breast cancer patient by Kaplan Meier plotter (KM plotter). We accessed KM plotter for the analysis of NIS and deiodinase with clinical results calculating relapse-free survival (RFS) and hazard ratio (HR).

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## METHODS

All data of this manuscript were mined from a public online open-access database (http: //kmplot.com/analysis/index.php?p=service&cancer=breast). KM analysis was performed to predict the prognostic values of deiodinase to analyse the relapse-free survival (RFS) and log-rank p values. We divided patients into ER+ve and ER-ve subgroups according to their ER status. Further KM plot analysis was performed to predict the prognostic values of DIO1-3 in the available Affymetrix based gene expression and RFS data from up to 801 ER-ve breast cancer cases and 2061 ER+ve breast cancer cases.[10] The relapsed free survival is defined as the length of time after treatment for cancer ends and the patient survives without reappearing of any signs or symptoms of that particular cancer. Calculating RFS is a way to estimate the efficacy of the treatment. We entered DIO1-3 into the database and after the validation of desired Affymetrix id; KM survival plots were extracted to analyse the RFS and log-rank p- values. Hazard ratios (HR) were computed at default cut off. Log-rank p- values were calculated and ≤0.5 were considered to be statistically significant. This type of analysis enabled us to directly perform in silico validation of DIO1-3 in number of breast cancer patients at risk were indicated below each KM plot.

## RESULTS

THs are important for mammary gland physiology and development.[11] Breast is a tissue which is under the physiological regulation via various hormones depending upon its development stage, lactation and involution as well as during breast neoplasia. In vitro TH induces proliferation of breast cancer cells.[12] Proliferative effect of T3 was blocked by co-treatment of oestrogen receptor antagonist.[13] As evidenced by significant crosstalk between these two hormones, we used KM plot data to analyse the DIO1-3 depending upon the ER status of breast cancer (Table 1). Breast cancer can progress in hormone-dependent manner as well as hormone-independent manner. We assessed the prognostic value of DIO1-3 in breast cancer using www.kmplot.com for ER+ve breast cancer patients (n=2061), and for ER-ve breast cancer patients (n=801) (Table-1). We did not observe significant correlation of DIO1-3 expression in ER+ve breast cancer patients (Figure 1 (A-C)). DIO1 high expression was correlated significantly with RFS in case of ER-ve breast cancer patients, HR 0.72 (0.57-0.91), p=0.0046 (Figure 2A). DIO-2 high expression was also correlated significantly with RFS in ER-ve breast cancer patients, HR 1.31 (1.05-1.65), p=0.019 (Figure 2B). However, DIO-3 expression did not significantly correlated with RFS in case of ER-ve breast cancer patients (Figure 2C). We expanded analysis of DIO1-3 expression in the hormone responsive breast cancer patients that are subjected to endocrine therapy. The data belongs to 929 cases (Table 2). DIO3 expression correlated significantly to the RFS in ER+ve breast cancer patients with endocrine therapy HR 0.7(0.54-0.91), p= 0.0083 (Figure 3C). However, DIO-1 and DIO-2 expression were not significantly correlated with ER+ breast cancer patients with endocrine therapy (Figure 3A, 3B).

| Deiodinases | ER Status | Cases | HR 95% CI | p Value |
|---|---|---|---|---|
| DIO1 | Negative | 801 | 0.72(0.57-0.91) | 0.0046 |
| | Positive | 2061 | 0.98(0.83-1.15) | 0.78 |
| DIO2 | Negative | 801 | 1.31 (1.05-1.65) | 0.019 |
| | Positive | 2061 | 0.89 (0.76-1.05) | 0.16 |
| DIO3 | Negative | 801 | 1.02 (0.81-1.28) | 0.87 |
| | Positive | 2061 | 0.91 (0.77-1.07) | 0.26 |

***Table 1. Correlation and Prognostic Value of Deiodinase Expression with ER Status of Breast Cancer Patients***

| Deiodinases | ER Status | Treatment | Cases | HR 95% CI | p Value |
|---|---|---|---|---|---|
| DIO1 | Positive | Endocrine therapy | 929 | 0.86 (0.67-1.12) | 0.27 |
| DIO2 | Positive | Endocrine therapy | 929 | 0.83 (0.64-1.08) | 0.16 |
| DIO3 | Positive | Endocrine therapy | 929 | 0.7 (0.54-0.91) | 0.0083 |

***Table 2. Correlation and Prognostic Value of Deiodinase Expression with Therapeutic Intervention of Breast Cancer Patients***

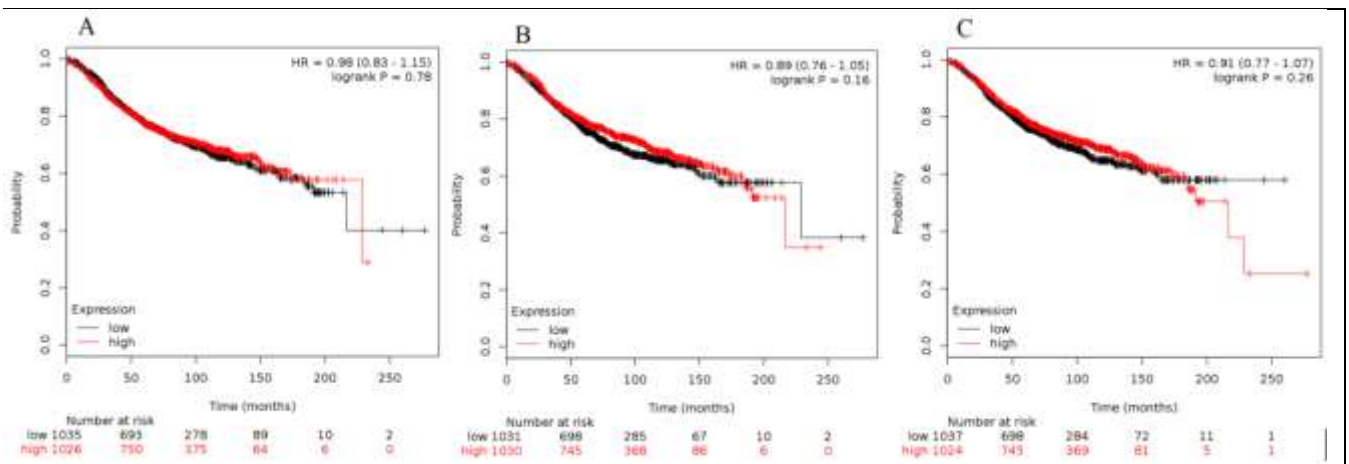


***Figure 1. Determination of Prognostic Value of Deiodinase Enzymes Expression Associated to Breast Cancer Patients with ER+ Status (n=2061 Patients)***

**(A)** RFS Curve Plotted for DIO1 Expression **(B)** RFS Curve Plotted for DIO2 **(C)** RFS Curve Plotted for DIO3. p-value and Hazard Ratios are Indicated on Top Right of Each RFS Plot

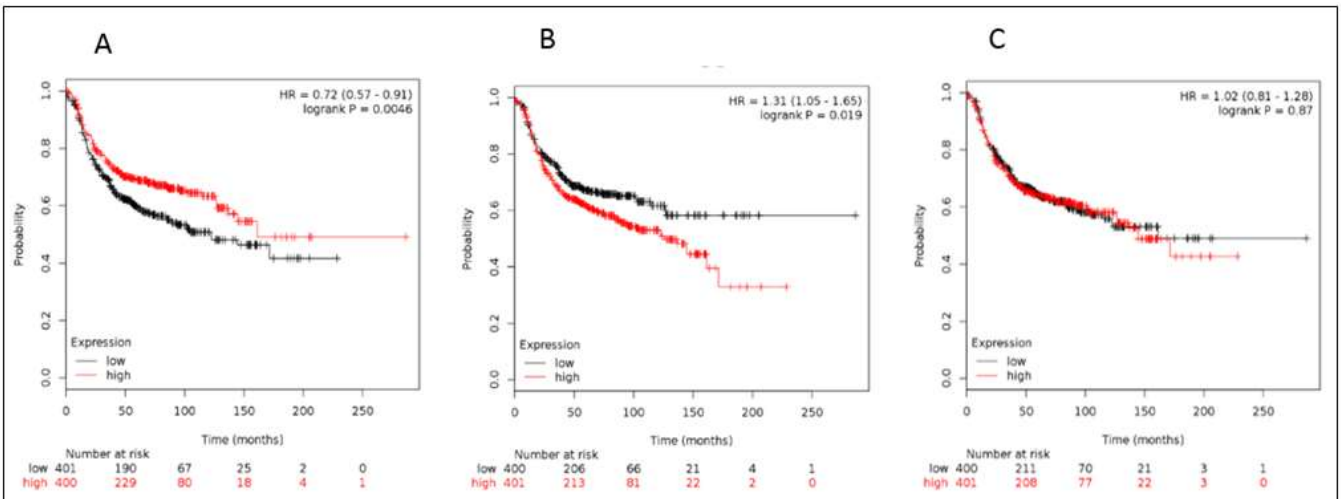


***Figure 2. Determination of Prognostic Value of Deiodinase Enzymes Expression Associated to Breast Cancer Patients with ER-Status (n=801 patients)***

**(A)** RFS Curve Plotted for DIO1 Expression **(B)** RFS Curve Plotted for DIO2 **(C)** RFS Curve Plotted for DIO3. p-value and Hazard Ratios are Indicated On Top Right of Each RFS Plot

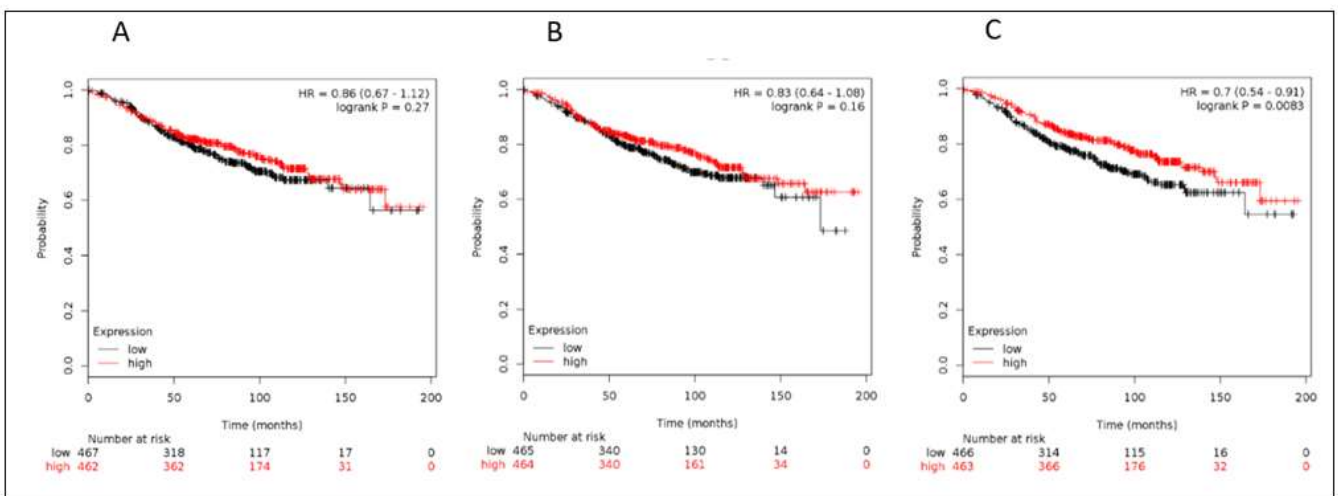


***Figure 3. Determination of Prognostic Value of Deiodinase Enzymes Associated to Breast Cancer Patients with ER+ Status Treated with Endocrine Therapy (n=929 Patients)***

**(A)** RFS Curve Plotted for DIO1 Expression **(B)** RFS Curve Plotted for DIO2 **(C)** RFS Curve Plotted for DIO3. p-value and Hazard Ratios are Indicated on Top Right of Each RFS Plot

## DISCUSSION

By using the data mining approach, we found that ER-ve Breast cancers, with high DIO1 and DIO2 expression significantly affect the RFS of Brest cancer patients. On the other hand, ER +ve breast cancer patients with hormone therapy, increased DIO3 expression result in worse RFS. We conclude that in some subgroups of breast cancer, Thyroid hormone activation and deactivation could play an important role.

THs play a critical role in tissue homeostasis and energy metabolism. Primarily their actions are exerted through binding with nuclear receptor and regulating the transcription of thyroid hormone-responsive genes. THs also modulate regulate metabolic processes and the energy consumption of the host. Cancer has been described as a disease of altered metabolism. THs genomic action and metabolic role is dependent on the expression of Deiodinases expression and it is conceivable that levels of deiodinases can be critically important for cancer development.

DIO1 expression is often found suppressed in cancer cells compared with normal tissue, for example, DIO1 expression and activity was not detected when compared with normal kidney cells.[14] Further DIO1 activity was also reduced in lung cancer,[15] Hepatic adenocarcinoma[16] and in Prostate cancer.[17] ER +ve DIO2 was decreased. DIO2 expression is upregulated in most tumours of glial cells.[18] TCGA data analysis has shown that DIO2 expression is upregulated in breast cancers compared to normal tissue.[9]

DIO3 express highly during embryonic development and cancer. Immortalized cell line in different tumours such as breast, colon, thyroid, and neuroblastoma express elevated D3 levels.[19,20] It remains to determine the impact of the alteration of availability of intercellular T3 and the role of dehydrogenase in this process still data from the ER+ breast cancer patient with endocrine therapy high expression of DIO3 provides that might lead to TH inactivation may provide survival advantage.

**CONCLUSIONS**

Deiodinases can modulate TH hormone and thus it's signalling at intracellular levels independent of its circulating levels. Changes in the expression and activity of DIO can lead to aberrant downstream signalling and contribute to tumour development. The detailed role of DIO is beginning to unravel, still; there is lack of data of these enzymes in various cancers. We conclude, based on the RFS data that DIO can be an important regulator of breast cancer development in context of ER status, and can also moderately influence the treatment outcome in ER +ve, hormone-responsive cancer subjected to endocrine therapy. More studies using breast cancer tissue levels and their enzymatic studies are warranted to further elucidate the underlying mechanism and to determine the extent of DIO role and breast cancer.

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