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Evaluation of the Effect of Oxaliplatin on XLOC_000303 (LINC01507), CRNDE and miR-181a-5p in HT29 Cell Line

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Abstract

Background: This study aimed to investigate the relationship between long non-coding RNAs *XLOC_000303*and *CRNDE* and *miR-181a-5p* and oxaliplatin in the HT29 cell line**.**

Methods and Results:HT29 and HUVEC cell lines were used in this study. Two subgroups were created in HT29 and Huvec cells: one treated with OXA and the other as a control group. Subsequently, we collected the cells and assessed their viability using the MTT assay and RNA isolation. The next step was to convert RNA into complementary DNA. Subsequently, the samples were evaluated using RT-qPCR for gene expression analysis.

Our findings indicated that the expression of *XLOC_000303*and *CRNDE* was significantly upregulated following oxaliplatin treatment in HT29 cells ($p<0.001$). Conversely, we observed the downregulation of *miR-181a-5p*($p = 0.004$). The AUCs were 1.00, 0.93, and 0.24, respectively. An inverse relationship was found between *CRNDE* and *XLOC_000303* expression levels and *miR-181a-5p*. These findings suggest that these lncRNAs may be involved in a potential regulatory network related to *miR-181a-5p*.

Conclusion: The inverse relationship between increased expression of *CRNDE* and *XLOC_000303*and decreased expression of *miR-181a-5p* in the HT29 cell line suggests the presence of a possible regulatory network in colorectal cancer. These findings suggest that the *CRNDE/XLOC_000303/miR-181a-5p* axis may play a potential role in regulating oxaliplatin response in HT29 cells. This axis needs to be confirmed by further functional studies. Targeting this axis offers a potential approach for developing novel therapeutic strategies to enhance chemotherapy efficacy.

INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide [1]. Despite surgical resection and adjuvant chemotherapy, the development of treatment resistance, particularly in advanced-stage disease, constitutes a significant clinical problem [2]. Therefore, elucidating the molecular mechanisms underlying oxaliplatin resistance is critical for identifying new therapeutic targets.

Oxaliplatin (OXA) is a platinum-based chemotherapeutic agent commonly used to treat CRC [3, 4]. It activates pathways involved in DNA damage repair and cell death [2, 5]. Although oxaliplatin-based regimens are standard treatments for CRC, patients may develop resistance to chemotherapy over time, which reduces the effectiveness of the treatment[6]. This resistance often arises from factors such as altered drug transport [7], enhanced DNA damage repair[8], evasion of apoptosis[9], and changes in gene expression regulation [10]. Understanding the molecular mechanisms behind chemotherapy resistance is important for improving treatment outcomes.

Long non-coding RNAs vary in length from 200 nucleotides to several kilobases[11], whereas miRNAs are shorter, consisting of 20–25 nucleotides [12]. Studies have shown that lncRNAs act as either tumor

suppressor genes or oncogenes, contributing to metastatic transformation through transcriptional, post-transcriptional, and epigenetic mechanisms [13]. Various studies have reported that miRNAs exhibit both oncogenic and tumor-suppressive properties. In particular, they have been found to act as epigenetic regulators during tumor development, invasion, and metastasis throughout the carcinogenesis process [14].

Differentially expressed colorectal neoplasia (*CRNDE*) is an lncRNA localized adjacent to the *IRX5* gene on chromosome 16q12.2 [15]. *CRNDE* is reported to play a role in cell proliferation, suppression of apoptosis, invasion, and metastasis; it also activates various oncogenic signaling pathways[16]. *CRNDE* lncRNA has been identified as an important regulatory molecule showing abnormally high expression in colorectal cancer and associated with tumor progression[17].

XLOC_000303 (LINC01507) is an intergenic long non-coding RNA approximately 210 kilobases in length, located on chromosome 9q21.31[18]. Observations suggest that *XLOC_000303* plays a important role in tumor formation across various cancers and could serve as a non-invasive biomarker [19]. However, there is insufficient information about its function, target genes, and related pathways.

MicroRNA-181a-5p *(miR-181a-5p)* is an important microRNA that regulates gene expression by suppressing the translation or degrading target mRNAs [20]. Alterations in *miR-181a-5p* expression levels have been observed in various cancer types, including colorectal cancer, and this molecule has been shown to play a role in key tumor processes such as cell proliferation, apoptosis, and metastasis [21–23].

In recent years, the roles of non-coding RNAs in cancer biology have become increasingly better understood [13, 19, 24]. Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) play a central role in the post-transcriptional regulation of gene expression [22, 25]. In this context, the "competitive endogenous RNA" (ceRNA) hypothesis proposes that different RNA types indirectly regulate gene expression by interacting with each other through common miRNA binding sites [26]. According to this model, lncRNAs can bind to specific miRNAs, thereby eliminating their repressive effect on target mRNAs. These networks are vital in shaping responses to chemotherapy. Yet, in the HT29 cell line model, the interactions between *CRNDE* and *miR-181a-5p*, particularly regarding their impact on oxaliplatin response, remain insufficiently explored. Additionally, the significance of *XLOC_000303* in this context is still unclear.

This study is designed to delve into the effects of oxaliplatin treatment on the interactions of *CRNDE*, *miR-181a-5p*, and *XLOC_000303* in the HT29 cell line. By clarifying the regulatory relationships among these molecules, we hope to enrich our understanding of chemotherapy resistance. Importantly, our research aims to uncover the potential of these non-coding RNAs as valuable biomarkers in the diagnosis and treatment of colorectal cancer, paving the way for improved patient outcomes.

MATERIAL-METHODS

Cell Lines and Treatment

The human cell lines used in this study were HT-29 *(*ATCC: HBT-38 *(P: 6*) and HUVEC (ATCC: CRL-1730, P: 9), all of which were obtained from Atlas Biotechnology (Ankara, Türkiye). Cell lines from the American Type Culture Collection (ATCC) were used in this study. To propagate the HUVEC and HT-29 cell lines, the cells stored in cryotubes were thawed and cultured according to ATCC's protocols recommended by the ATCC. HT29 (HTB-38) (P: 6) and HUVEC (CRL-1730) (P: 9) cell lines were cultured in 25 cm² cell culture dishes (07-8025, Biologix). They were grown in DMEM (Dmem-ha, Capricorn) medium with 20% FBS (FBS-HI-11B, Capricorn), 1% penicillin-streptomycin (PS-B, Capricorn), and 1% L-glutamine (GLN-B, Capricorn). The cultures were incubated at 37°C with 5% CO_2 and then passaged. The cells in the flasks were examined under a microscope during incubation. We monitored their growth and studied them when they reached the appropriate size and number. We then applied oxaliplatin (brand: Yunbang Bio., CAS. No: 61825-94-3, Cat. No: YB52615, MF: C8H14N2O4Pt, MW: 397.29, solubility: 5 mg/mL in water) to the cell lines at different time points and concentrations.

Experimental Procedures

MTT Cell Viability Test

The MTT assay was used to test the viability of HT29 and HUVEC cells treated with oxaliplatin. The cells were counted using Trypan blue in a 96-well plate with 2×10^4 cells in each well and were then seeded. Each well had a total volume of 100 µL, including 10% Fetal Bovine Serum. The cells were treated with oxaliplatin at concentrations of 2.5, 5, 10, 25, 50, 100, and 200 µM. The treatment durations were 6, 12, 24, 48, and 72 h. Before treatment, the solution was diluted to a final volume of 100 µL. After treatment, 10 µl of MTT solution (Biotium, MTT cell viability test kit, Catalog No: 30006) was transferred to the wells. Thereafter, the plates were placed in an incubator at 37°C for 4 h. Finally, each well contained 200 µl of DMSO (Sigma-Aldrich, D2650). We performed measurements at 570 nm using an ELISA microplate reader (Heales MB-530; Shenzhen, China). MTT analysis generated a graph showing the relationship between cell viability rates and oxaliplatin concentrations. We calculated the ideal oxaliplatin dose at the following time points: 6, 12, 24, 48, and 72 h. The optimal IC_{50} concentration of oxaliplatin for the HT29 cells was 48 µM (IC_{50}:47,99 µM and exposure for 48 hours). Each experiment was performed three times.

Bradford Protein Analysis (ELISA Analysis)

The Bradford Protein Analysis protocol was applied as recommended by the manufacturer. Total protein concentration was measured using the Bradford Protein Assay Kit with Bovine Serum Albumin (BSA) as the standard, thus indicating the total protein presence [27]. Preparation of standards involves mixing 1.5 mL of Bradford reagent with 30 µL of BSA, followed by incubation at room temperature for 5 minutes to

establish a standard curve. Sample handling involves placing 5 µL of each sample into microplate wells, followed by the addition of 200 µL Bradford reagent and incubation for 5 minutes. Absorbance measurement is performed at 595 nm using a plate reader. Blank correction is applied by subtracting the blank absorbance from all sample readings. Protein concentrations are calculated based on the standard curve generated from BSA standards.

Cell Preparation,miRNA and Total RNA Extraction

After culturing the cells to reach the desired number, we seeded 6 × 104 cells into 6-well plates, with each plate containing three replicates. The cells were then removed from the incubator after a 24-hour incubation period. The cells were centrifuged at 4000 rpm for 10 min at 4°C. Total RNA (GeneAll Biotechnology Hybrid-R kit (catalog no. 305 – 101, Seoul, Korea) and microRNA were extracted using the Trizol reagent(GeneAll RiboEx kit (catalog no. 301-001, Seoul, Korea). The obtained RNA samples were stored at -80°C until further use. The concentration and purity of RNA were measured using a Nanodrop (Qubit 4 Fluorometer, Invitrogen).

cDNA Synthesis

The miRNA and total RNA were reverse transcribed to synthesize cDNAs using an A.B.T. cDNA Synthesis Kit with RNase Inhibitor (High Capacity, Catalogue No. C03-01-05, Turkey). Next, the cDNA synthesis reactions were carried out in a thermocycler (ProFlex 96-Well PCR System, Thermo Fisher Scientific, Singapore) as follows: 10 minutes at 25°C, 120 minutes at 37°C, and 5 minutes at 85°C. The synthesized cDNA samples were stored at -80°C.

Q-RT-PCR Expression Analysis

qRT-PCR was carried out using QuantStudio5 real-time PCR systems (Applied Biosystems™, Thermo Fisher Scientific, Singapore). The SYBR Green master mix (ABT, A.B.T.™ 2X SYBR Mastermix, Catalog No: Q03-01-05, Turkey, and ABT, A.B.T.™ 2X miRqGreen MasterMix (with ROX), Catalog No: Q04-02-01) was utilized in the process of PCR amplification. GAPDH and hsa-mir-16-5p were employed as internal controls. The real-time PCR amplification mix was first activated at 95°C for 5 minutes. Then, denaturation at 95°C for 15 seconds, followed by annealing and extension at 60°C for 60 seconds. Finally, extension at 72°C for 30 seconds, for 40 cycles. The primer pairs used are summarized in Table 1. The primer's specificity was confirmed through the implementation of a melting curve analysis. Each PCR reaction was performed three times, and the mean cycle threshold (Ct) data for the oxaliplatin-treated and control groups were obtained using cycle threshold settings. Expression levels between groups were analysed using the 2-ΔΔCt method.

Table 1
Primer sequences for amplification of the studied ncRNAs (5'→ 3')

Target gene	Forward sequence (5’→3’)	Reverse sequence (5’→3’)
CRNDE	TTTCCGGAGTAGAGCCCTTG	CTCCTCCTTCCAATAGCCAGT
XLOC_000303	ACCTTGCAACACTCTCTGGA	TCCAACCTCTTCAGCTCCAG
GAPDH	AGGGCTGCTTTTAACTCTGGT	CCCCACTTGATTTTGGAGGGA
hsa-miR-181a-5p	AACATTCAACGCTGTCGGTGAGT	CATCTTCAAAGCACTTCCCT
hsa-miR-16-5p	TAGCAGCACGTAAATATTGGCG	CGAGGAAGAAGACGGAAGAAT

Statistical Analysis

The data were analysed statistically and graphically using SPSS 21.0 software (SPSS Inc., Chicago, IL) and GraphPad Prism 8.0.2. The Shapiro-Wilk test was employed to assess the conformity of quantitative variables to a normal distribution. The data were found to be normally distributed ($P > 0.05$). All data were obtained from a minimum of three independent experiments. The data is presented as the mean ± standard deviation (SD). An unpaired t-test was used to estimate statistical differences between the two groups. A two-way analysis of variance (ANOVA) was applied. The differences between the groups were evaluated using Tukey's multiple comparison post hoc test. IC_{50} values were calculated using a nonlinear regression (4PL dose-response) model in GraphPad Prism, based on MTT experimental data.The sensitivity and specificity values of the target genes in the control groups treated with oxaliplatin were evaluated using the area under the curve (AUC) and receiver operating characteristic (ROC) analyses. Data with a p-value less than 0.05 were considered statistically significant.

RESULTS

Cell Viability (MTT and Bradford’s-ELISA assays)

The MTT method was used to determine the time and dose-dependent cell viability by applying oxaliplatin to HUVEC and HT29 lines at varying doses. The graphs showing the applied dose and cell viability are in Fig. 1. In this study, the IC_{50} doses were determined as 48 µM (48 hours, 47.99 µM) in HT29 cells. In the HUVEC and HT29 cell lines, the control and oxaliplatin dose groups were analysed in triplicate. Protein measurements were performed using Bradford's assay and ELISA methods. As a result of the evaluation of protein concentrations, no statistically significant difference was found between the HT29 oxaliplatin-treated and HT29 control groups ($P > 0.05$). However, in the HUVEC control group, it was determined that the final protein concentrations were increased compared to the HUVEC oxaliplatin-treated group, and a statistically significant difference was found between them ($P < 0.05$) (Fig. 2). The table presents the mean and standard deviation values of the data (Table 2).

Table 2

Shows a comparison of the expression levels of *miR-181a-5p, CRNDE* and *XLOC_000303* in oxaliplatin-treated HUVEC and HT29 cells, as well as in the control group. The data have been presented as the mean ± standard deviation. Two-way ANOVA with Tukey's multiple comparison post hoc test. CI: confidence interval for mean; SD: standard deviation; HUVEC: Human Endothelial Cell; HT29:HT29 Colorectal Cancer Cell; $p < 0.05$ consider significant, *$p < 0.05$ **$p < 0.001$

Gene	Group	Mean + SD	95%	Sig. (P)
XLOC_00303	HUVEC Oxaliplatin	1,69 ± 0,16	1,2798-2,0976	0,000**
	HUVEC Control	0,85 ± 0,81	0,6496-1,0544	
	HT29 Oxaliplatin	2,78 ± 0,11	2,5131-3,0383	
	HT29 Control	0,92 ± 0,04	0,8210-1,0257	
CRNDE	Huvec Oxaliplatin	1,65 ± 0,35	0,7902-2,5178	0,004*
	HUVEC Control	0,97 ± 0,11	0,7072-1,2408	
	HT29 Oxaliplatin	1,85 ± 0,32	1,0536-2,6471	
	HT29 Control	0,10 ± 0,09	0,7704-1,2209	
miR-181a-5p	HUVEC Oxaliplatin	0,56 ± 0,13	0,2315-0,8831	0,000**
	Huvec Control	1,43 ± 0,13	1,0965-1,7549	
	HT29 Oxaliplatin	0,66 ± 0,15	0,2833-1,0360 CI	
	HT29 Control	1,09 ± 0,01	0,8488-1,3259	

Evaluation of ncRNAs Expression Levels in HT29 and HUVEC Oxaliplatin, Compared to the Control Group

Our results showed statistically significant differences in the expression levels *of miR-181a-5p* (*P = 0.001*), *CRNDE* (*P = 0.004*), and *XLOC_000303* (*P = 0.001*) both between and within groups (Table 3). The *XLOC_000303, miR-181a-5p*, and *CRNDE* ncRNAs (Fig. 3A, B, C) were significantly different in the oxaliplatin group compared to the control group. In the HT29 cell line, oxaliplatin administration resulted in an increase in CRNDE and XLOC_000303 levels and a decrease in miR-181a-5p. The highest expression levels of miR-181a-5p, *CRNDE*, and *XLOC_000303* were recorded in the HUVEC control and HT29 oxaliplatin-treated groups, respectively. The lowest expression level of *miR-181a-5p* was observed in the oxaliplatin-treated HUVEC group. No statistically significant differences were observed among the genes in the HT29 control cells. In HUVEC control cells, there was a statistically significant decrease in *miR-181a-5p* levels ($P < 0.05$) (Fig. 3D).

Table 3
ELISA results are presented as mean and standard deviation values of final protein concentrations.

Groups	HT29 Oxaliplatin Group	HT29 Control Group	HUVEC Oxaliplatin Group	HUVEC Control Group
Mean	0,52	0,50	0,47	0,59
Std. Deviation	0,02	0,06	0,02	0,06

ROC and AUC Analyzes

The area under the ROC curve (AUC) was calculated to determine whether candidate non-coding RNAs could be used as a biomarker. A comparison was made between the HT29 treatment group and the control group, resulting in the identification of the following AUCs of ROC: The following were found to be statistically significant: *XLOC_000303* (95% CI: 1.000–1.000; p = 0.013; AUC = 0.100); *CRNDE* (95% CI: 0.757–1.000; p = 0.033; AUC = 0.926); and *miRNA-181a-5p* (95% CI: 0.000–0.510; p = 0.196; AUC = 0.241). The results of this study are presented in Fig. 4.

DISCUSSION

In this study, the effects of oxaliplatin administration on the HT29 cell line were investigated in the non-coding RNAs. Expression analysis results showed that the HT29 treatment with the oxaliplatin group had significantly increased expression levels of *XLOC_000303* ($p < 0.001$) and *CRNDE (*$p < 0.001$), while *miR-181a-5p* ($p = 0.004$) decreased (Fig. 3). This expression pattern is thought to offer important clues regarding the molecular regulatory mechanisms accompanying the oxaliplatin response. In addition, analyses regarding their potential as biomarkers revealed that *XLOC_000303* (AUC = 1.00; p = 0.013) and *CRNDE* (AUC = 0.926; p = 0.033) had high discriminatory power in the HT29 OXA group (Fig. 4). It is increasingly understood that lncRNAs play critical roles in cancer biology, and particularly in chemotherapy response. In this context, *CRNDE* has been reported to exhibit oncogenic properties in various cancer types and is associated with cell proliferation, invasion, and treatment resistance. The increased *CRNDE* expression observed in this study suggests that this molecule may play a role in cellular adaptive response processes to oxaliplatin administration. Moreover, the observed decrease in miR-181a-5p levels suggests that the tumor suppressor functions of this miRNA may be suppressed. miRNAs are known to play a crucial role in regulating gene expression and maintaining cellular homeostasis by inhibiting translation or degrading target mRNAs. Therefore, a decrease in miR-181a-5p levels could reduce apoptotic responses in cells or increase proliferative signals, thereby contributing to oxaliplatin resistance.

There are a limited number of studies on the role of XLOC_000303 (LINC01507) in tumorigenesis. In a study on cervical cancer cases, it was reported that *XLOC_000303* (*LINC01507*) was upregulated and could be used as a potential biomarker to predict tumor formation in cervical cancer [28]. In another study, researchers observed that LINC01507 was significantly upregulated. The researchers suggest that

LINC01507 may be a valuable predictor of prognosis and treatment responses in patients with clear cell renal cell carcinoma [29]. In our previous study on plasma samples from colorectal cancer patients, we found that *XLOC_000303* was upregulated, showed expression differences between stages, and was a protective factor in metastasis formation. Consequently, we suggested that it could be a non-invasive biomarker in colorectal cancer cases [30].

The *CRNDE* has been identified as an oncogene in a variety of cancers [31]. The upregulation of *CRNDE* has been shown to inhibit apoptosis in CRC cells while promoting cell proliferation, invasion, and migration [32]. A lot of studies have shown that *CRNDE* overexpression significantly promotes cancer cell proliferation. Specifically, this expression pattern is associated with Wnt/β-catenin, PI3K/AKT/mTOR, and Ras/MAPK signaling pathways and contributes to increased proliferation [33, 34]. X. Bai et al. found that *CRNDE* acts as an oncogene via the PI3K/Akt signalling pathway, and that its expression is positively correlated with invasion and lymph node metastasis stage in gastric cancer [35]. In another study, Han et al. found that *CRNDE* expression is upregulated in CRC patients, suggesting that it may play a role in regulating CRC development. In CRC cells, *CRNDE* has been shown to promote the expression of *miR-181a-5p*, thereby inhibiting Wnt/β-catenin signaling [36]. Our previous study showed that *CRNDE* was upregulated in the plasma of colorectal cancer patients [37]. Zhang *et al*. found that *CRNDE* is associated with chemosensitivity in gastric cancer tissues and cells [38]. Gao et al. reported that *CRNDE* overexpression increased CRC cell resistance to oxaliplatin [39]. Furthermore, an increasing number of studies are demonstrating that *CRNDE* contributes to tumor development by targeting microRNAs via a ceRNA mechanism. *CRNDE* may also contribute to resistance against oxaliplatin-based chemotherapy [39–41].

miR-181a-5p is a member of the *miR-181a* microRNA family and has been the focus of research due to its regulatory role and altered expression in various diseases, including cancer. Studies have shown that the expression levels of *miR-181a-5p* can be either upregulated or downregulated in different tumors. These findings highlight its significant role in regulating tumorigenesis by post-transcriptionally repressing targeted genes [21, 22]. Many studies have indicated that miR-181a-5p plays a role in the development and progression of cancer, acting as an oncomiR or tumour inhibitor in multiple types of cancer [42, 43]. An investigation of patients with pancreatic ductal carcinoma showed that plasma *miR-181a-5p* could be used as a specific biomarker to monitor responses to FOLFIRINOX treatment [44]. Studies have shown that *miR-181a-5p* inhibits tumour growth in colorectal cancer by affecting the Wnt/β-catenin signalling pathway. Cell proliferation and 5-FU sensitivity were also found to be inhibited, with apoptosis being promoted [20, 45]. Another study has shown that lncRNA ANRIL can act as a sponge for miR-181a-5p, thereby preventing apoptosis and radio-sensitivity in colon cancer cells [46]. A similar study has shown that *miR-181a-5p* is associated with liver metastasis in colorectal cancer [47]. Zhao et al. observed a decrease in *miR-181a-5p* levels in cisplatin-resistant cells[47]. The research indicates that *miR-181a-5p* can effectively reduce the IC_{50} value of these cells, reverse cisplatin resistance, and increase sensitivity to platinum drugs except in ovarian cancer [41]. Another paper was conducted to investigate the effect of *LINC01232* on colon adenocarcinoma cells. The findings revealed that *miR-181a-5p* was downregulated and showed a negative correlation with *LINC01232* [24].

Consequently, the study data show an inverse relationship between CRNDE and miR-181a-5p. This finding appears consistent with the ceRNA model, suggesting that lncRNAs interact with microRNAs via a "sponge" mechanism. It's thought that CRNDE may suppress miR-181a-5p, weakening its regulatory effect on target genes and potentially altering cellular responses. However, more functional studies are needed to confirm this relationship at the molecular level.

According to the findings, although the role of XLOC_000303 has not yet been fully elucidated, the observed increase in its expression level is noteworthy. The simultaneous increase in XLOC_000303 and CRNDE suggests that similar signaling pathways may regulate these two transcripts or may work together in related biological functions. Furthermore, when these data are examined together with the decrease in miR-181a-5p levels, it can be suggested that XLOC_000303 may also play a role in the regulation of microRNAs and potentially be involved in the ceRNA network. However, additional research is required to validate this hypothesis, including direct interaction analyses and experimental validation of functions.

CONCLUSION

These findings suggest that the *CRNDE/XLOC_000303/miR-181a-5p* axis may play a potential role in regulating oxaliplatin response in HT29 cells. Specifically, the evaluation of the *CRNDE/XLOC_000303/miR-181a-5p* axis within the context of a competitive endogenous RNA network has the potential to enhance our understanding of the molecular mechanisms underlying chemotherapy resistance in colorectal cancer. However, further experimental studies are required to elucidate the functional role of this axis. Consequently, it is hypothesised that these findings could provide a potential basis for the development of targeted therapy strategies in the future.

Limitations of the study

The current study has some limitations. First, the analyses are limited to a single cell line, the HT29 model. So the findings need to be supported and validated in different cellular systems and by a larger patient population and clinical data. Furthermore, the study remained at the gene expression level; protein-level validation and functional analyses were not performed. Therefore, further molecular and cellular studies are crucial to definitively establishing the proposed mechanisms.

Declarations

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Ethical Declaration: None (This study was conducted with cell lines and did not require approval from the ethics committee.)

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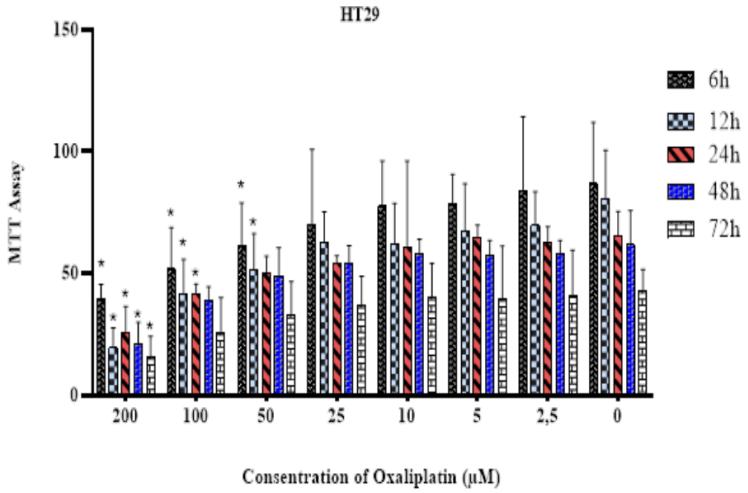
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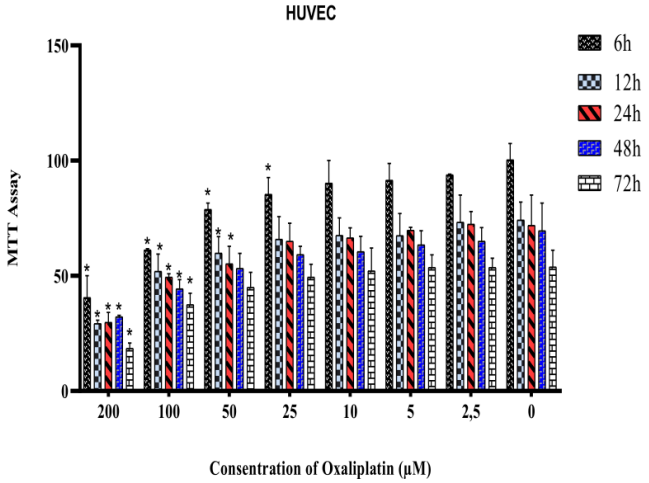
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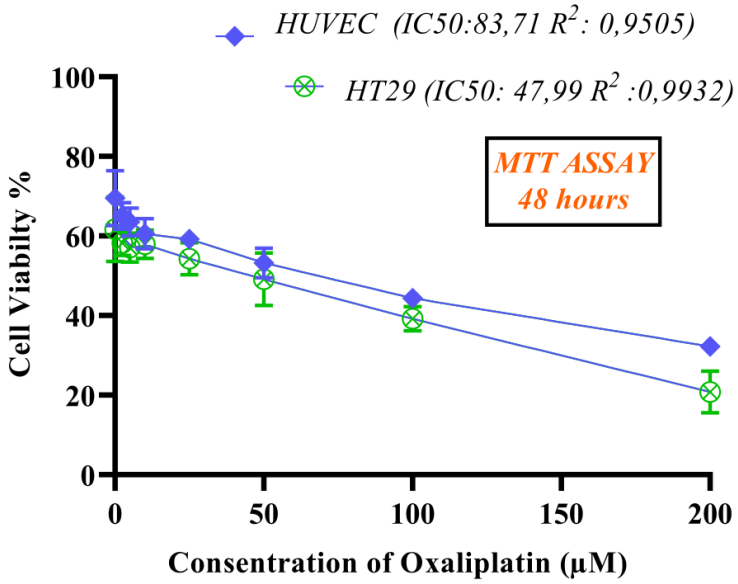
Figures



a



b



c

Figure 1

a-b. Time and dose dependent cell viability rates of HT29 and HUVEC cells c- IC_{50} values of HT29 and HUVEC cells at 48 hour is shown.

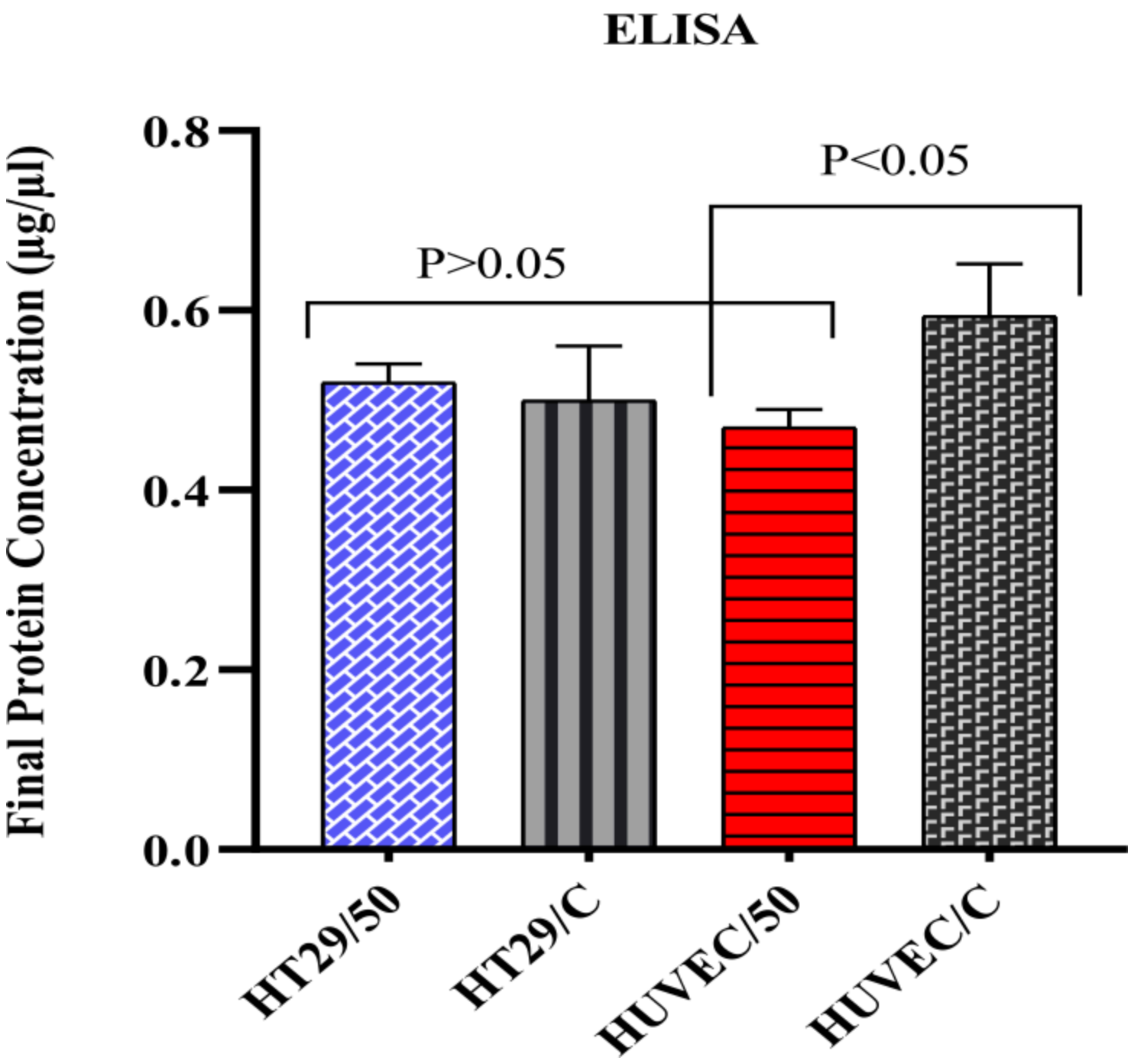


Figure 2

Total protein concentrations were compared using the Bradford protein assay protocol (ELISA technique was used). P<0.05: significant, P>0.05:no significant

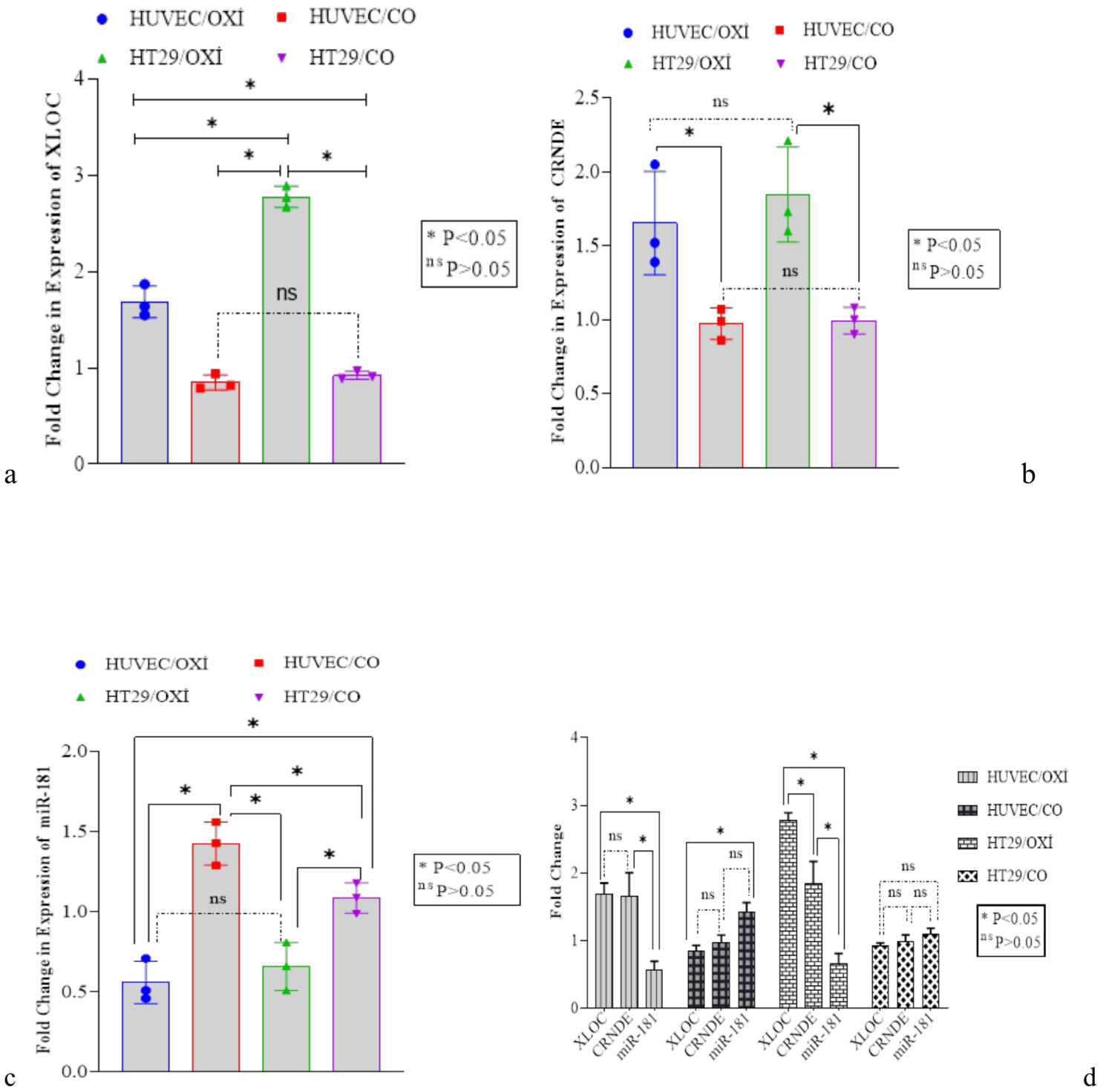


Fig 3

Figure 3

a. *XLOC_000303* expression level in HT29 and HUVEC cell lines b. *CRNDE* expression level in HT29 and HUVEC cell lines c. *miR-181a-5p* expression level in HT29 and HUVEC cell lines. d. The changes between *XLOC_000303, CRNDE* and *miR-181a-5p*genes in HUVEC and HT29 cell lines. OXİ: Oxaliplatin, CO:Control, *P<0.05(Analyzed by Tukey's multiple comparison post hoc test) OXİ:Oxaliplatin, Co:Control (Analyzed by Tukey's multiple comparison post hoc test)

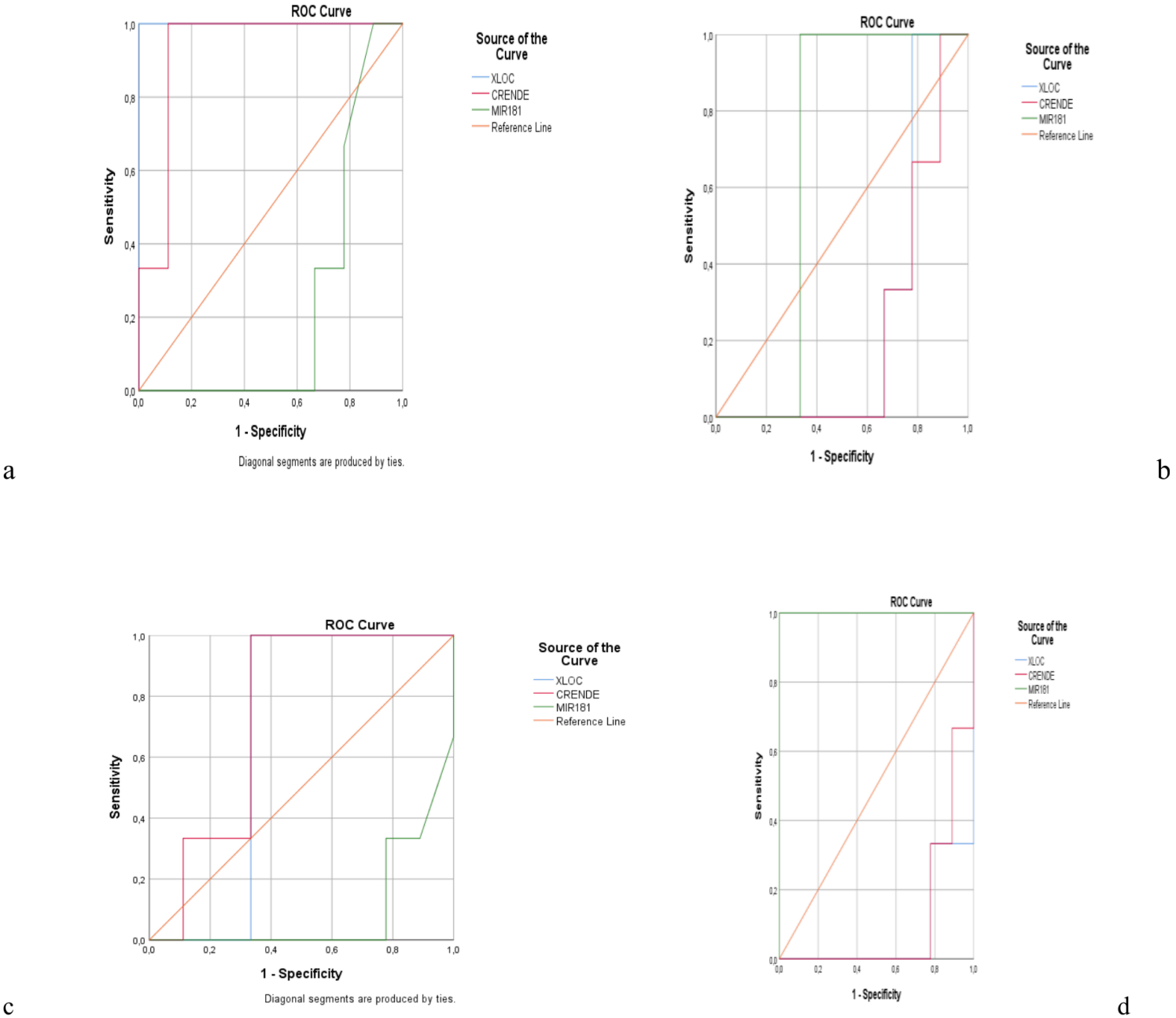


Fig 4

Figure 4

Receiver operating characteristic (ROC) curves for evaluating the diagnostic power of ncRNAs: *XLOC_000303, CRNDE, and miR-181a-5p*. ROC curve and the area under the curve were calculated to determine the sensitivity and specificity CRC cell line as a clinical biomarker a) HT29 OXA cells b) HT29 control cells c) HUVEC OXA cells d) HUVEC control cells