§4. Discussions

Ovarian carcinoma is the fifth most common cancer of women in the Unite State and has the highest mortality among the gynecological malignancies in western countries [73,74]. Ovarian cancer accounts for 5% of all cancer deaths in western countries. In the majority of cases, the disease has already spread beyond the pelvic cavity at the time of diagnosis [74]. Although in last years the successful introduction and the development of cisplatin-based chemotherapy resulted in an improvement of patients' survival, the percentage of recurrent disease is high even in those who achieve a complete response to cisplatin –based chemotherapy. Moreover, about 80% of patients with advanced stage of this disease die within 5 years. At present, the prognostic characterization of patients with ovarian cancer based on clinic-pathological parameters seems to be inadequate, since patients with similar clinic- pathological characteristics often experience different prognosis [77]. Therefore, the identification of biological factors related to tumor aggressiveness could be relevant in order to identify patients with different prognosis and chance to response to the chemotherapy and other affiliated therapy method. Moreover, it allows the selection, at the initial diagnostic time, of the high-risk patients needing the more aggressive therapy or an alternative treatment, and a closer follow-up.

Among the biological parameters proposed as possible prognostic factors in ovarian cancer, much attention has been focused on the endocrine factors and especially on the steroid hormone [77,78]. Epithelial ovarian cancer is considered to arise from the ovarian surface epithelium, which shares a common embryonic origin with epithelia of Mullerian duct-derived tissues (fallopian tube, endometrium, endocervix) [68,77]. Moreover, the ovary is the main source of estrogen genesis in women. Epidemiological data and most of *in vitro* studies had demonstrated that, similar to breast cancer and endometrial cancer, the ovarian cancer cell biology could be influenced by the hormone signal pathway. Estrogen taken as oral contraceptives during pre-menopausal years offer the protection, but when used post-menopausally as hormone replacement therapy, elevates the cancer risk [76]. However, conflicting data have been reported about the possible clinical role of ERs in ovarian cancer [78]. There should be some other pathways closely associated with E-ER signal transduction and regulation of endocrine biology in the ovarian cancer cell. A family of potential candidate biomarkers is the orphan receptors family, estrogen receptor-related receptor (ERRs). The studies on the expression and function of ERRs in the ovarian cancer may provide another explanation on the complex

endocrine biology of ovarian cancer cell.

4.1 Estrogen-ER signal pathway and ovarian caner

Estrogen is very important for human development, sexual differentiation and maintenance of both the female and male reproductive system. Endogenous and exogenous estrogen may play important roles in the pathogenesis and progression of ovarian cancers, yet their mechanism of action reminder unclear. Abnormal estrogen level has implicated as risk factor for ovarian carcinoma in previous documents [2,3,68,75-77]. The ovary is the main source of estrogen in women, the estrogen being formed in granulose cell from androgenic precursors derived from the theca. According to the concept “incessant ovulation”, the formation of inclusion cysts at the ovarian surface following each ovulation could expose the lining epithelium to abnormally high levels of gonadotrophins or estrogen. The deregulation of normal patterns of proliferation and differentiation of ovarian epithelium could represent the first step for genetic alternations to accumulate and to be transferred to daughter cells, thereby proceeding along a multi-step path of molecular oncogenesis [77]. Genomic functions of estrogen are mediated by classical nuclear receptors, ERα and β, which are ligand-regulate transcription factors activating the target gene expression by recruiting co-regulators to specific DNA elements [1,4,44,54]. Although there are conflicting data reported, the possible clinical role of ER in ovarian carcinomas. Many experimental observations confirm the hypothesis of an involvement of estrogen-estrogen receptor signal pathway in the oncogensis of ovarian cancer.

4.1.1 ERα and ERβ are highly expression in ovarian cancer

Estrogen receptors, both the ERα and ERβ subtypes, were found expressing in normal human ovaries, benign ovarian tumor, borderline and malignant ovarian tumors, as well as in primary cultures of normal human ovarian surface epithelial cells (HOSE) and established ovarian cancer cell lines [68,75-78]. The functional characteristics of ERs in terms of affinity, specificity, and capacity appeared to be similar in malignant tumors compared with benign ovarian tumors or normal ovarian tissue. However, the concentration of ER in ovarian carcinomas appears to be generally higher than it is in benign ovarian tumors or normal ovaries [77]. In this work, about 69.6% (23/33) patient with ovarian cancer and 41.7% (5/12) normal ovarian samples can be detected an ERα positive-expression by ICH staining and Q-PCR analysis. ERβ positive expression was

detected in 17 cases of 33 ovarian cancer samples (51%) and 6 cases of 12 normal ovarian epithelial cells (50%). Similar to previous reports, an increasing positive-expression rate and mRNA levels were found in ovarian cancer compared with normal ovaries [75-77,79]. However, the positive-expression rate of hERβ was no significant difference between the ovarian cancer group and normal ovaries group. The high variability in the percentage of ERs positivity may be related to the different assay methods [77].

4.1.2 The role of ERα and ERβ in ovarian cancer

The role of ER status as possible prognostic parameters in the patients with ovarian cancer has been debated for years. There is some evidence that expression of ER does not play a prognostic role in ovarian cancer. On the contrary, considered by the other reporters, the ERs positive-expression patients with ovarian cancer seemed to have a longer median overall survival [77]. The date from *in vitro* research also showed that ERs have a relatively poor value in the prediction of ovarian tumor cell sensitivity to treatment with tamoxifen or medroxyprogesterone acetate [77]. For example, the ovarian cancer cell line SKOV-3 was detected with ERα and ERβ positive-expression but resistant to the hormonal therapy [80]. Moreover, although estrogen and estrogen receptors are involved in the genesis and progression of ovarian disease, only a small number of ovarian carcinomas are responsive to the relatively non-toxic anti-estrogen therapy such as tamoxifen and progestin. Tamoxifen has been the most widely used as anti-estrogen therapy for relapsed ovarian cancer. However, in a Cochrane database report, a response rate in ovarian cancer treatment with tamoxifen varied from 0%-56% [81,82]. A phase II study of hormonal therapy with Letrozole for relapsed epithelial ovarian cancer demonstrated no association between the ERs expression and response to the treatment [81]. On the contrary, Bowman *et al.* reported statistically significant association between high ER expression and both disease stabilization as measured by CT scan and CA125 response/stabilization [83]. Furthermore, analysis of the CA-125 endpoint indicated a highly significant trend between increasing expression of ER and likelihood of CA-125 stabilization or response. These conflicting phenomena strongly suggest that there should be some other pathways to regulate the classical signal pathway of estrogen-ER-estrogen responsive action in ovarian cancer cells. Considering the growing body of knowledge about ERRs, the expression of ERRs may provide another estrogen-like stimulus in the over-proliferation and tumorgenesis of ovarian cell.

4.2 Expression of ERRs in ovarian cancer

As potential endocrinal biomarkers in ovarian cancer, estrogen receptor-related receptors (ERRs) have exhibited biochemical and transcriptional activities that are similar to, yet distinct from the classical ERs. ERRs can also modulate the transcription of at least some genes that are estrogen responsive and/or implicated such as pS2, aromatase and lactoferrin [19,40-42,66,72]. As an initial attempt to determine whether these orphan nuclear receptors might be associated with ovarian cancer, I detected the expression of the major isoforms members of ERRs family and ERs family in ovarian cancer cell lines and primary ovarian cancers. To discuss the relationship between these two nuclear receptor families, I also compared their different expression pattern in the ovarian cancer samples as well as in the normal ovaries. Most ovarian cancers are mainly derived from ovarian surface epithelium [68,75,76,79]. However, the normal OSE is only a single layer of cells. Similar to various work groups [75,76,78,84], I compared the expression of ERRs mRNA and protein in ovarian cancers and normal ovaries that included the ovarian surface epithelium and stroma. To our knowledge, this study was the first research about the expression of hERRs in the established ovarian cancer cell lines as well as ovarian cancer tissues.

4.2.1 Expression of exogenous ERRs in ovarian cancers

Previous studies have demonstrated the detection of ERRs mRNA in mouse adult tissue by Northern Blot or In Situ Hybridization [9,16,33]. The detail expression of human ERRs in the ovarian tissues is not clear yet. At first, the subcellular localization of hERRα was examined. The ovarian cancer cells SKOV-3 and OVCAR-3 were transfected with hERRα-GFP reporter plasmid and observed under the cofocal scan microscope. After the successful transfection, the cells will produce a fusion protein. Using the double color confocal scan and single color confocal scan, the fusion protein can report the subcellular distribution of hERRα. Excited by a 480n illumination, the exogenous fusion protein translated from hERRα-GFP plasmid can produce a green fluorescent signal without any staining. Compared with the different green auto-fluorescent signals observed in the cell nucleus and cytoplasm, we concluded that this exogenous hERRα was mainly expressed in the cell nucleus. Some studies also reported that the exogenous fusion ERRα protein was a kind of nuclear receptor by the yeast two-hybridization method [9-11,16].

The cells transfected with the HA-tag-hERRγ plasmid will express HA-hERRγ fusion

proteins. To detect this exogenous fusion protein, the antibodies only anti to the HA tag epitope but not anti to the hERRγ protein were used in the Western-Blot analysis. Since the HA tag epitope could not be produced by the human ovarian cancer cell lines SKOV-3, OVCAR-3 and ES-2 themselves, there will be no cross- binding between the antibodies and any other homological epitope. Therefore, the immunoblot detected in the membrane will report the distribution of exogenous HA-hERRγ protein [12]. Results form the Western-Blot showed that the expression of exogenous recombinant HA-hERRγ protein could be detected in the nuclear protein extraction but not in the cytoplasmic protein extraction. It also means that the exogenous HA fusion hERRγ protein is a kind of nuclear protein.

4.2.2 Expression of endogenous ERRs in ovarian cancers

Furthermore, as soon as the commercial antibodies anti-to human ERRα, ERRβ, ERRγ, were available in October 2004 [71], an immunocytochemistry examination on the *in vitro* cultured cells and immunohistology examination on the *in vivo* ovarian tissues were also performed. Results from our study, both hERRα and hERRγ express abundantly on ovarian cancers. This increasing of mRNA expression on ovarian cancers means the ERRα and ERRγ do have some association with the development of ovarian cancer. The antibodies anti to hERRs protein were chiefly binding to the cell nucleus. However, results from the *in vivo* examination indicated that there is also some weakened expression of hERRs protein in the cell cytoplasm. The results from the expression of hERRα-GFP reporter protein and HA-tag protein demonstrate that the human ERRs are chiefly expressed in the cell nucleus. In general, the detection in the expression of exogenous and endogenous hERRs protein has demonstrated that hERRs are chiefly located in the nucleus of ovarian cancer cells. A similar result was also reached in others studies: Suzuki et al. [71] reported a nuclear location of hERRα-1 in breast caner tissue; Cheung et al. [85] reported a subcellular nuclear distribution both of exogenous and endogenous hERRα protein in human prostate cells and tissues. It suggests that hERRs protein may have an important role in the cell nuclear biology.

In my study, 57.6% of ovarian cancers were observed with expression of hERRα protein and 48.5% ovarian cancers with expression of hERRγ protein, which indicates that high levels of hERRα and hERRγ might be associated with ovarian cancer. By the quantitative PCR analysis, the hERRα mRNA level were significant increasing in the ovarian cancers than in the normal ovaries ($p<0.05$). Different with the classical ERs, which are

ligand-activated transcriptions, the ERRs do not bind to natural estrogen. High expression of hERRα may provide another pathway to stimulate cell overgrowth in ovarian cancer. Our results suggested that the human ERR family might also be involved in the tumorogenesis via binding to the ERRE of oncogenes in ovarian cancer. However, this hypothesis needs to be validated by additional studies on the function of ERRs in ovarian cancer. Other studies showed that hERRα could not be activated nor repressed by natural estrogen, agonists or antagonists of estrogen [26,27,28]. Since the hERRα is resistant to the classical inhibitor of estrogen, this may explain why ovarian cancers respond poorly to anti-estrogen therapy based on only blocking the estrogen-ER signaling pathway. The observed conflicting clinical role of ER in ovarian cancer may be the result from the hERRα influence. It also suggests that ERRα's phosphorylation status may have predictive value in assessing the effectiveness of the selective estrogen receptor modulator (SERM) adjacent therapies in ovarian cancer.

It contrast to abundant expression of ERRα and ERRγ, ERRβ and its isoformal seem poor in the ovarian cancer and normal ovaries. ERRβ is present early in the developing placenta, a subset of cells in extra-embryonic ectoderm destined to develop the chorion and only found in a very low concentration in a few specific rat tissues (kidney, heart, hypothalamus, hippocampus cerebellum and rat prostate) [11,20,36]. In mice lacking ERRβ, trophoblast stem cell differentiation is impaired and the placenta fails to develop normally [35,36]. The research on the function of ERRβ is limited by their poor expression. The above data in our study underlines that ERRs, especially the hERRα and hERRγ, are important in ovarian cancer. They might contribute to the development of ovarian cancer independent of the classical estrogen signaling.

4.3 The association between ERs and ERRs in ovarian cancer

It is known now that the ERRs family includes three isoforms: ERRα, ERRβ and ERRγ, and each member has several different transcripts. Previous studies focus primary on the interaction of ERα and ERβ in the ovarian cancer [86,87,88,89]. We have shown for the first time that human estrogen receptor-related receptors α, β and γ expression pattern with ERα and ERβ in ovarian cancer cell lines, normal ovaries as well as ovarian cancer samples. The transcription activity of each ERR depends on the promoter, the particular cell lines and the presence of ERs. For example, whereas ERRα stimulates ERE-dependent transcription in the absence of ERα Hela cells, it down-regulates

estradiol-stimulated transcription in ERα positive human mammary carcinoma MCF-7 cells via an active mechanism of repression [28, 41-44,66].

In our study, both of the ovarian cell lines SKOV-3 and OVCAR-3 were found to have high expression of ERRα, which have been confirmed to express two subtypes of ER. However, the cell line OVCAR-3 was considered as a tamoxifen treatment sensitive ovarian cancer cell line, while SKOV-3 was demonstrated resistant to the anti-estrogen treatments [85], Results form this study also shown that in all ovarian samples with any subtypes of ER (24 cases), there are high co-expression of ERRα (70.8%,17/24). Reports from other studies have shown that ERα and ERRα were competitively bound to the DNA responsive elements and co-activators in a cell-specific, promoter-specific manner [23,28,41-44]. ERRs share target genes, co-regulatory proteins and DNA binding sites of action with the ERs. ERRs were found to bind as a monomer, with a high affinity binding site containing the extended half-site sequence 5'-TCA-AGGTCA-3', which can be seen in the ERE, ERRE (estrogen receptor related-receptor responsive element), SFRE (steroid factor-1 receptor responsive element). Since increasing expression of ERRα can affect the function of ERs, they will repress the E-ER-ERE mediated target gene transcriptions in ER-positive cells. A high ERα/ERRα ratio may indicate a high function of ERα, on the other hand, a lower ERα/ERRα may indicate a suppressed function of ERα by an over-expression of ERRα. Compared with the different mRNA expression levels of hERRα and hERα, a high ERα/ERRα ration (>1.0) was observed in the OVCAR-3 cells but not in the SKOV-3 cells (ERα/ERRα<1.0). Moreover, a high ERRγ/ERRα ratio was also observed in the ovarian cancer cell lines. Compared with the normal ovaries, the ERα/ERRα ratio seemed to be decreasing in the ovarian cancers. This result may explain why only 7%-18% responsive rate of anti-estrogen therapy, which target on the blocking of ER and E binding, in the ovarian. According to our results, a new hypothesis has arisen that it should be the ERRα but not ERα as the really therapy target on the anti-estrogen resistant ovarian cancer, or, in another words, the endocrine therapy should be targeted on both of ERs family and ERRs family.

ERRs serve as constitutive regulators of some genes with an ERE/ERRE on their promoter. For example, the ERRs can modulate the transcription of some genes that are estrogen responsive such as pS2, aromatase, osteopontin and lactoferrin. Zhang and Teng reported that hERRα1, the major isoforms of the human ERRs, could activate transcription of some estrogen responsive genes and exert ER-like function via binding to ERE or ERRE sites [41-44,66]. More reports pointed out that hERRα-1 can activate or

repress ERE-regulated transcription in a cell type-dependent manner, repressing the ERE mediated transcriptions in ER-positive MCF-7 cells while activating the ERE mediated transcriptions in ER-negative Hela cells. It suggests that there is a key role of ERRs in the regulation of the classical estrogen-estrogen receptor-ERE signal pathway, and estrogen related cancers such as breast cancer, endometrial cancer and ovarian cancer. However, the mechanisms of this cross-talking between these two receptor subfamilies are still not clear. In ER-negative tumors or ones with high ERRα levels, ERRα become a major regulator of ERE-containing genes.

4.4 The potential role of ERRs as ovarian cancer biomarker

As this is just a beginning on the understanding of ERR function, only few clinical researches on ERRs have been reported. To discuss the potential use of ERRs as ovarian cancer biomarkers, the expressions of ERRs were analyzed combining with multi-parameters. Serum CA-125 and the malignant of ovarian was also confirmed by the correlation analysis. Therefore, the association between the serum CA-125 of patient with ovarian cancer and the expression of human ERRs were investigated. The ovarian cancer patients with ERRα positive-expression (both the protein and mRNA level was positive-expression, see the definition listed in the **3.6.1**) showed higher serum CA-125 levels than ERRα negative cancers ($p<0.05$), which indicated a potential poor prognosis. Moreover, combining analysis with other clinic pathological data, hERRα positive-expression ovarian cancer patients were associated with disease that is more aggressive: short median overall survival time ($p<0.05$) and more advanced FIGO stage ($p<0.05$). Furthermore, the hERRα positive-expression group showed a trend with poor cell differentiation and more ascites, although there is no statistical significance between the hERRα positive-expression group and hERRα negative-expression group. The results from my study strongly suggest that hERRα play an important role in ovarian cancer. The ovarian cancer patients with high hERRα expression level may associate with a poor prognosis. Considering about this modest sample size, a large-scale sample size should be performed to further discuss the role of hERRα as an unfavorable tumor biomarker.

On the other side, both the expression of hERRβ and hERRγ were not correlated with the serum CA-125 levels. More interesting, the hERRγ positive ovarian cancer patients have a significantly longer progression-free survival ($p<0.05$) than the ovarian cancer

with hERRγ negative expression. Ariazi and his colleagues were the first to use real-time Q-PCR to assess the importance of ERRs in human breast cancer by comparing with their mRNA profiles with clinically established pathological indicators and mRNA profiles of ERs and ErbB family members [70]. They found that an increased ERRα level was associated with aggressive breast tumor behavior and increased ERRγ levels was associated with benefit clinical course. Suzuki confirmed this result in his study: the ERRα-1 is a poor prognosis marker in breast cancer [71]. We get this same result from our study on ovarian cancers. A slight difference with their result is that they found ERRγ was over expressed on 75% of the tumors. In our study, 45% of ovarian cancers show a positive of ERRγ. This is may be the different distribution of ERRγ in different tissues, and may be the result of a more sensitive response to the SERM treatment in breast cancer.

In contrast to the abundant expression of hERRα and hERRγ, two hERR isoforms (hERRβ-1 and hERRβ-2) seem to be poorly expressed in both of the ovarian cancer and normal ovarian tissues. ERRβ is present in the early developing placenta in a subset of cells in extra-embryonic ectoderm destined to develop the chorion and only found in very low amounts in specific rat tissues [11,35,36]. The knowledge about hERRβ is limited due to low and restricted expression. The data of our study suggest that hERRα and hERRγ may be important in ovarian cancer and may contribute to the development and progression of ovarian cancer. In our study, hERRα is a tumor marker associated with poor prognosis and hERRγ seems to be a tumor marker for favorable prognosis in ovarian cancer. The determination of the status of human ERRs expression in ovarian cancer may improve the hormonal therapy and the prognostic evaluation of ovarian cancer. However, large-scale prospective and retrospective studies are needed to establish whether ERRs expression is indeed of practical utility as a prognostic predictor.

4.5 Are the ERRs new therapy targets in the hormone related cancer?

The factors that determine ERRα's transcriptional activity still need to be identified. Results from in vitro research has shown that ERRs were potential therapy targets for endocrinopathic cancer [35,49,50,72]. Based on my work, I suppose the same hypotheses: human ERR, especially the hERRα and hERRγ, are potential therapy targets in ovarian cancer. Ovarian cancer can be considered as a hormone-dependent

cancer; however they are not sensitive to hormonal therapy. Select estrogen receptor modulators (SERM) such as tamoxofen act as anti-tumor agents by inhibiting the binding of ER and estrogen agonist, mainly in breast tumors. Although anti-estrogen therapy is well established in ER-positive breast cancer, ovarian carcinomas respond quite poorly to relatively non-toxic hormonal therapy. Hormonal therapy for recurrent epithelial ovarian cancer has resulted in uneven but consistent response [81,82,83,84]. In general, 70% ovarian tumors are ER positive expression [68,75-77], only about 7% to18% primary ovarian cancers respond to the anti-estrogen therapy [77,81,82,83,84]. A systematic Cochrane review of 623 patients has documented a moderate activity of tamoxifen in relapsed ovarian cancer, the response rate varied from 0%-56% [82]. It suggests that only target on the blocking E-ER binding is not enough in the anti-estrogen treatment in ovarian cancer, although ovarian cancers are also high expression of hERs. One probable reason is the influence from a functional expression of ERRs, especially the expression of hERRα.

Results from some previous studies have demonstrated that ERRs were independent with classical E-ER-ERE signal pathway: ERRα could not be either activated or repressed by natural estrogen, agonists or antagonists of estrogen [11,28]. Therefore, the high expression of ERRα in ovarian cancers may explain why the ovarian cancers respond poorly to the anti-estrogen therapy based on the blocking E-ER signal pathway. Concerned about a high expression of hERRα protein and mRNA in the patients with ovarian cancers (60% samples with mRNA level >200 copies/μl and >50% samples with protein expression positive), the hERRα may be also a potential therapy target for ovarian cancer. High-level expression of hERRα was associated with the poor prognosis; moreover, it also was confirmed as an independent factor. It is an interesting topic to study whether decreasing expression of hERRα will benefit to the patients with ovarian cancer. Though there hasn't been any special inhibitor for hERRα, Yang reported that two oragnochlorine pesticides, toxaphene and chlordane, were antagonists in *in vitro* cell lines experiments. Target on the hERRα and/or hERα therapy may has an encourage anti-tumor effectively on these estrogen related cancers. However, this hypothesis needs further experiments to be performed. The complicated therapeutic results of SERM using as anti-tumor treatment in the ovarian cancer might be explained by a very complicated ERs and ERRs status, which is still unclear.

In contrast to ERRα, both ovarian cancer and normal ovaries showed high expression of hERRγ. Moreover, the activity of hERRβ and hERRγ can be inhibited by

4-hydrotamoxifen (4-OHT), which is an active component of tamoxifen, one of the mostly commonly used SERMs in the therapy of breast cancer [49,50]. Compared with other SERMs, 4-OHT is the only compound tested that showed significant activity in cell based assays. This could suggest that ovarian cancer patients with hERRγ expression may be more sensitive to the treatment with SERMs. Indeed, the key discovery that 4-OHT is an inhibitor or probable ligand for human ERRβ and ERRγ predicts novel and unexpected use for current SERMs [28,49,50]. The design of new SERMs that could special block that ERRα activity or both ERα and ERRα activity should be explored [28, 46,66].

In summary, the discovery of human estrogen receptor-related receptors provided another target to try endocrinal therapy, which may benefit to the anti-tumor treatments in estrogen-related cancers. However, these hypotheses need a lot of *in vivo* and *in vitro* trials of ovarian cancers target on the expression of hERRs. More research is necessary to reveal the biological significance of these molecules in ovarian cancer cell physiology and ovarian tumor genesis. This different characteristic of ERs and ERRs may prove to be one of the keys to understand the anti-estrogen resistance in ovarian tumors. Based on the given results from this work, further studies should be involved the association between the difference ERRs status and the chemotherapy. Whether the ERRα is associated with a more advanced clinical stage ovarian cancer or a more refractory ovarian cancer is a potential study goal.

Previous studies have mostly focused on the ERα and ERβ. We have first shown that human estrogen receptor-related receptors α, β and γ expression pattern with ER α and ER β in ovarian cancer cell lines as well as ovarian cancer samples. In our study, SKOV-3 was found highly expression of ERRα, which has been shown to express two subtypes of ER but is estrogen and anti-estrogen resistant. In addition, Ovcar-3 was a sensitive cell line to estrogen-stimulate or anti-estrogen treatments. More than that, on our result shown that there are only 15% samples with only ERs expression, but in all ovarian samples with any subtypes of ER, there are highly expressions of ERRα (70.8%, 17/24). Since expression of ERRα can affect on the function ERs, they will repress the ERE mediated transcriptions in ER-positive cells. This result may explain why only 7%-18% response rate of anti-estrogen therapy on the ovarian. According to our results, a new hypothesis was promoted that it should be the ERRα but not ERα as the really therapy target on the anti-estrogen resistant ovarian cancers.

It would be of interest to determine and compare the different levels of both ERs and ERRs in tumors that do and do not respond to anti-estrogen therapy. The different

characteristics of ERs and ERRs may prove to be one of the keys to understand anti-estrogen resistance in ovarian tumors. Further studies, such as assessing whether ERRα is functional in ovarian tumors and correlating both ERRs proteins and ERs proteins with the clinic outcome and response to anti-estrogen should be the focus of future research goal.

§5. Conclusions

This study described here is an initial investigation in the potential role of human estrogen receptor-related receptors in ovarian cancer. Expression of human ERRα seems a novel biomarker associated with poor prognosis and expression of human ERRγ may indicate a more sensitive responsive to SERM treatment. However, the role of human ERRβ was limit discussed in this work for its weak expression in ovarian cancer and normal ovarian tissues. Some major conclusions are made as follows. In general, these conclusions need a bigger scale study to further confirmed.

- ERRs are chiefly expressed in the cell nucleus.
- Both of hERRα and hERRγ may play an important role in ovarian cancer.
- Over-expression of hERRα is closely associated with more advanced clinical stage of ovarian cancer and more aggressive malignant biological behavior.
- ERRα and ERRγ are potential targets of ovarian cancer therapy.