Research Journal of Medical Sciences 1 (2): 74-79, 2007

Cholangiocarcinoma: An Estrogen-Dependent Cancer?

[1,2]Domenico Alvaro, [1]Maria Grazia Mancino, [3]Paolo Onori,
[4]Antonio Franchitto and [4]Eugenio Gaudio
[1]Division of Gastroenterology, University of Rome, "La Sapienza", Rome, Italy
[2]University of Rome "La Sapienza", Polo Pontino, Latina, Italy
[3]Department of Experimental Medicine, Section of Human and Clinical Anatomy,
State University of L'Aquila, L'Aquila, Italy
[4]Department of Anatomy, University of Rome "La Sapienza", Rome, Italy

INTRODUCTION

Cholangiocarcinoma is a malignant tumor, which originates from cholangiocytes, the epithelial cells lining the biliary tree (Malhi and Gores, 2006). Cholangiocarcinoma is one of the cancers with the worst prognosis with the 5-years median survival rate only 7-8% and the median survival time only seven months (Malhi and Gores, 2006; Lazaridis and Gores, 2005, 2006). Recent epidemiological studies show an increasing incidence and prevalence over the last 3 decades (Malhi and Gores, 2006; Lazaridis and Gores, 2005, 2006). It has been calculated that the incidence correlates to 2,000-3,000 cases per year in the United States, doubling in South East Asia and Eastern Europe, probably in relation with the different distribution of risk factors (Malhi and Gores, 2006; Lazaridis and Gores, 2005, 2006). Nevertheless, intra-hepatic and extra-hepatic cholan-giocarcinoma show different epidemiological characte-ristic (Malhi and Gores, 2006; Lazaridis and Gores, 2005, 2006). In fact, the intra-hepatic form is increasing in incidence and mortality, whereas the extrahepatic form is stable (Lazaridis and Gores, 2005). Unfortunately, the late diagnosis causes this cancer to be surgically unresectable in more than 50% of the cases (Lazaridis and Gores, 2006). Furthermore, common chemotherapies are virtually ineffective and therefore the mortality rate for cholan-giocarcinoma has been virtually unaffected by medical attempts in the last years (Malhi and Gores, 2006; Lazaridis and Gores, 2005, 2006).

In the last years, progress in cell and cancer biology lead to important advances in the therapeutic approaches to different neoplasms (Fava *et al*., 2006; Cordera and Jordan, 2006). This is especially true for hormone-dependent cancers including neuroendocrine tumours as well as prostate and breast cancer (Cordera and Jordan, 2006). In this latter case, the demonstration of estrogen-dependence and effectiveness of Estrogen Receptors (ER) selective modulators radically changed the clinical course of the disease (Cordera and Jordan, 2006). Cancer biology of cholangiocarcinoma is still scarcely known and biological treatment applied only in single cases (Fava *et al*., 2006). Recent studies suggest that estrogens play a role in the cancer biology of cholangiocarcinoma (Alvaro *et al*., 2006; Mancino *et al*., 2006). The aim of this study is to revise recent studies dealing with the role and mechanisms by which estrogens modulate growth and proliferation of cholangiocarcinoma cells.

ESTROGENS, ER AND THE LIVER

Estrogens induce the proliferation of normal and neoplastic cells expressing Estrogen Receptors (ER) by both genomic and non-genomic pathways where multiple signalling transduction pathways are involved (Eagon *et al*., 1985, 1996; Blum and Cannon, 1998). ER are tran-scription factors that exist in an inactive apoprotein state either in the cytoplasm or in the nucleus (Eagon *et al*., 1985, 1996; Blum and Cannon, 1998). Once bound by estrogens, ER undergoes conformational changes allowing the receptor to bind DNA elements in target gene promoters and activate transcription (Eagon *et al*., 1985, 1996; Blum and Cannon, 1998; Fisher *et al*., 1984). Two main ER subtypes are known, ER-α and -β (Kuiper *et al*., 1998; Mosselman *et al*., 1996; Barkhem *et al*., 1998; Paige *et al*., 1998; Wakeling *et al*., 1991). Depending on the expression of ER subtypes, estrogens may activate different signalling pathways, which are via homodimers in cells expressing only one ER subtype or via hetero-dimers in cells expressing both ER subtypes (Kuiper *et al*., 1998; Mosselman *et al*., 1996; Barkhem *et al*., 1998; Paige *et al*., 1999; Wakeling *et al*., 1991). ER-α and -β also showed different sensitivity to antiestrogens (Barkhem *et al*., 1998; Wakeling *et al*., 1991). ER are expressed in hepatocytes, where estrogens may play a role in the process of growth and regeneration

Corresponding Author: Domenico Alvaro, University of Rome, "La Sapienza", via R. Rossellini 51, 00137 Rome, Italy

(Francavilla *et al*., 1989; Migliaccio *et al*., 2002). After partial hepatectomy, for example, ER expression in hepatocytes increases together with their translocation to the nucleus, where they induce DNA synthesis and allow restoration of normal liver mass (Francavillla *et al*., 1989). Estrogens are also involved in liver growth in the neonate and long-term administration in the adult induces enlargement of liver mass (Francavillla *et al*., 1989). Recently, we showed that estrogens play a key role in modulating cholangiocytes proliferation by activating both a direct (genomic) and indirect (non genomic) pathway and involving intracellular cascade (Ras/Raf/Erk) that are typical of Growth Factors, Including IGF1 (Insulin-like Growth Factor 1) (Alvaro *et al*., 2000, 2002 a, b, 2004, 2005; Gigliozzi *et al*., 2004).

ESTROGENS AND CHOLANGIOCARCINOMA

In different type of cancers, estrogens also act by inducing the synthesis and release of growth factors and cytokines other than synergizing the effects of growth factors and, in doing so, favour the growth, proliferation and spreading of tumour mass (Wimalasena *et al*., 1993). In addition, a recent emerging concept is that estrogens may affect neo-angiogenesis, which is a fundamental step in cancer growth, involving multiple mechanisms and complex loops of growth factors and cytokines (Ferrara and Kerbel, 2005).

We have recently investigated the expression of ER in human intrahepatic cholangiocarcinoma and human cholangiocarcinoma cell lines and evaluated the role of estrogens in the modulation of neoplastic cell growth (Alvaro *et al*., 2006). Eighteen biopsies of patients with intrahepatic cholangiocarcinoma, presenting as a single mass lesion within the liver, were investigated. While cholangiocytes of normal liver were negative, 18 out of 18 patients with intrahepatic cholangiocarcinoma showed an intense positive staining for both ER-α and -β. Most importantly, immunohistochemical positivity involved more than 80% of the cholangiocarcinoma cells with a staining involving both the cytoplasm and nucleus, the latter being indicative of activated receptors (Alvaro *et al*., 2006). The patients investigated were post-menopausal females or males, all with normal bilirubin serum levels and this should exclude that serum estrogen levels have influenced the immunohistochemical expression of ER. Very importantly, in spite of differences in sex, age and degree of tumour differentiation, the ER immunohistochemistry showed very homogeneous features suggesting that the intense positive staining for ER represents typical characteristics of cholangiocarcinoma. When compared with benign cholangiocyte proliferation associated with human cholangiopathies (Alvaro *et al*., 2004), the immunohistochemical expression of ER-ß was similar between cholangiocarcinoma and cholangiocyte proliferation associated with primary biliary cirrhosis, primitive sclerosing cholangitis and alcoholic cirrhosis while, in contrast, the expression of ER-α was 4 fold higher, suggesting a role in cancer progression. Recent studies, in fact, suggest that a high ER-α/-ß ratio characterizes malignant versus benign proliferation of ER expressing cells (Bardin *et al*., 2004; Diel, 2002; Lau *et al*., 2000; Girault *et al*., 2004; Sampson *et al*., 1997). ER-α expression was always linked with a positive modulatory effect of estrogens on proliferation and growth (Bardin *et al*., 2004; Diel, 2002; Lau *et al*., 2000; Girault *et al*., 2004; Sampson *et al*., 1997). The ER-ß is highly heterogeneous, being constituted by different splice variants (Lau *et al*., 2000). However, recent studies indicate an antiproliferative effect of this receptor subtype (Lau *et al*., 2000). Recently, a decreased expression of ER-ß (mRNA and proteins) or an increased ratio ER-α/ER-ß has been described in tumour versus normal tissues, including ovary, prostate, colon and breast cancers (Bardin *et al*., 2004; Diel, 2002; Lau *et al*., 2000; Girault *et al*., 2004; Sampson *et al*., 1997). In these tissues, neoplastic transformation and progression have been associated with an up-regulation of ER-α and down-regulation of ER-ß, indicating an opposite role of the two receptor subtypes in modulating estrogen-dependent neoplastic cell growth (Bardin *et al*., 2004; Diel, 2002; Lau *et al*., 2000; Girault *et al*., 2004; Sampson *et al*., 1997). However, further studies aimed at investigating the isoforms of ER-ß preferentially expressed during neoplastic cholangiocyte proliferation should be performed.

To evaluate, the role of estrogens on malignant cholangiocyte proliferation, we investigated different cholangiocarcinoma cell lines and found that only the cell line derived from human intrahepatic cholangiocarcinoma, HuH-28, expressed the protein (western-blot) of ER-α and -β as did the human cholangiocarcinoma biopsies (Alvaro *et al*., 2006). In previous studies, ER were detected by immunoprecipitation in cholangiocarcinoma cell lines (OZ and SK-ChA-1) but immunohistochemical analysis was negative in both cell lines (OZ and SK-ChA-1) and biopsies of human cholangiocarcinoma (Sampson *et al*., 1997; Nash *et al*., 2003). However, at variance with the present study, antibodies not specific for ER subtype were used (Sampson *et al*., 1997; Nash *et al*., 2003). HuH-28 cells also express IGF1 and IGF1-R with a level of protein expression (western-blot) similar to cell lines derived from colon or hepatocellular carcinoma where IGF1 is thought to play a key role in modulating neoplastic growth (Diel *et al*., 1999; Surmacz, 2003). Therefore, we used HuH-28 cells to evaluate the

role of estrogens and IGF1 in modulating neoplastic cell growth. By studying a human intrahepatic cholangiocarcinoma cell line (HuH-28), that similarly to intrahepatic cholangiocarcinoma, over expresses ER-α, IGF1 and IGF1-R, we showed that IGF-1 and estrogens stimulate proliferation with additive effects (Alvaro *et al*., 2006). Both ER antagonists (Ici 182,780) and IGF1-R blocking antibody (αIR3) inhibit the proliferative effects of estrogens and IGF-1 on HuH-28 cells (Alvaro *et al*., 2006). Moreover, IGF-1 and estrogens exert additive effects on HuH-28 cell proliferation where IGF1-R seems to play a primary role (Alvaro *et al*., 2006). We have shown, in fact, that IGF1-R blocking antibody (αIR3) partially inhibited the effects of 17β-estradiol and transfection of HuH-28 cells with IGF1-R antisense oligonucleotides caused a marked impairment of HuH-28 cells proliferation (90% decrease of PCNA expression) (Alvaro *et al*., 2006). In estrogen sensitive tissues, a cross-talk between IGF1 and estrogens play a major role in the modulation of cell proliferation, where estrogens act at several points of the IGF signal transduction pathway (Kehlert *et al*., 2000; Adesanya *et al*., 1999; Stephen *et al*., 2001; Zhou *et al*., 2001; Kassem *et al*., 1998; Cardona-Gomez *et al*., 2001). Estrogens may not only regulate the expression of IGF1-R and IGF1-binding proteins but also that of crucial down-stream proteins including IRS1, the IGF1-R tyrosine kinase main substrate (Kahlert *et al*., 2000; Adesanya *et al*., 1999; Stephen *et al*., 2001; Zhou *et al*., 2001; Kassem *et al*., 1998; Cardona-Gomez *et al*., 2001). Finally, the signaling activated by estrogns and IGF1 may converge at different common transduction pathways, including ERK and phosphatidylinositol-3 kinase/Akt pathway (Kahlert *et al*., 2000; Adesanya *et al*., 1999; Stephen *et al*., 2001; Zhou *et al*., 2001; Kassem *et al*., 1998; Cardona-Gomez *et al*., 2001). In proliferating HuH-28 cells, we found that both IGF1 and 17β-estradiol increase the protein expression of phosphorylated (p)-ERK1/2 and p-AKT and a further increase occur when the two substances are added together, suggesting convergence of the two agents into these signaling pathways (Alvaro *et al*., 2006). In addition, ER-α and IGF1-R, once activated co-precipitate and, their activation is potentiated by their coupling (Kahlert *et al*., 2000). Nevertheless estrogens can modulate other important mechanisms involved in cholangiocarcinoma growth such as COX-2 (Mali and Gores, 2006; Lazaridis and Gores, 2005, 2006; Bruegg-emeier and Diaz, 2006; Hermenegildo *et al*., 2006). These new evidence highlights the role of estrogens and IGF1 in regulating the growth of human cholangiocar-cinoma (Fig. 1) and suggests that therapeutic strategy based on the modulation of ER and/or IGF1 system could be helpful for the management of this cancer.

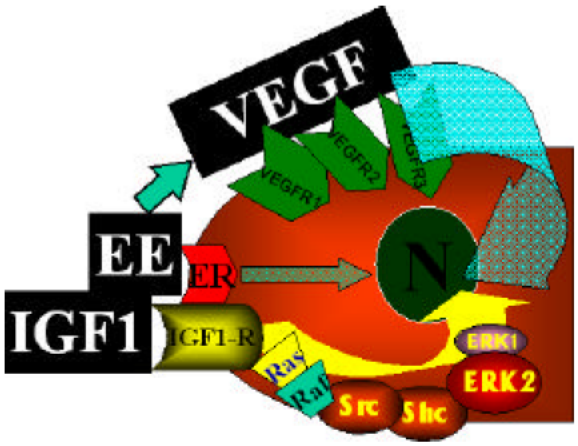

Fig. 1: Estrogens, IGF1 and VEGF Interplay in the modulation of cholangiocarcinoma proliferation. Estrogens induce the proliferation of cholangiocarcinoma cells by multiple mechanisms including: a) direct effect; b) the confluence with IGF1 and VEGF in common intracellular pathways (Ras/Raf/SRc/Shc/ERK1-2 pathway) and; c) the induction of synthesis and release of IGF1 and VEGF which in turn, through an autocrine mechanism, further enhance proliferation

ESTROGENS, CHOLANGIOCARCINOMA AND CANCER NEO-ANGIOGENESIS

Neo-angiogenesis is a fundamental step in cancer growth that involves multiple mechanisms and complex loops of growth factors and cytokines (Ferrara and Kerbel, 2005). Recently, a number of different observations indicate that estrogens play a major role in the induction of neo-angiogenesis in estrogen-sensitive cancers (Hyder, 2002; Ruohola *et al*., 1999; Nakamura *et al*., 1999). The Vascular Endothelial Growth Factor (VEGF), which is a main player in the mechanisms underlying neo-angiogenesis, has been considered to be responsible for the angiogenic action of estrogens in both normal and neoplastic tissues (Hyder, 2002; Ruohola *et al*., 1999; Nakamura *et al*., 1999). A number of different obser-vations indicate that estrogens play a major role in the induction of neo-angiogenesis in estrogen-sensitive cancers (Hyder, 2002). VEGF, which is a main player in the mechanisms underlying neo-angiogenesis, has been considered to be responsible for the angiogenic action of estrogens in both normal and neoplastic tissues (Hyder, 2002; Ruohola *et al*., 1999; Nakamura *et al*., 1999). We recently investigated whether estrogens promote the proliferation of HUH-28 cells by inducing the VEGF

system. HuH-28 cell line derived from human intrahepatic cholangiocarcinoma express VEGF-A, VEGF-C and their receptors (VEGFR-1, -2, -3). Estrogens markedly enhanced the expression of VEGF and VEGF-R in HuH-28 cells in association with stimulation of cell proliferation (Mancino *et al*., 2006). We first showed that HuH-28 cells express the 3 different VEGF receptors (-1, -2, -3) other than proteins for VEGF-A and VEGF-C (Mancino *et al*., 2006). This is in keeping with what is demonstrated in surgical samples of human intrahepatic (Park *et al*., 2006) and extrahepatic cholangiocarcinoma (Ogasawara *et al*., 2001) which showed immunohistochemical positivity for VEGF. As far as intrahepatic cholangiocarcinoma is concerned, more than 70% of surgical samples showed imunohistochemical positivity for VEGF-C, which represented an independent and important prognostic factor, suspected to play an important role in the lymph node metastasis (Park *et al*., 2006). As far as cell lines are concerned, VEGF but not VEGF receptors were found in three different cell lines derived from extrahepatic biliary tract (Nakamura *et al*., 1999; Park *et al*., 2006; Ogasawara *et al*., 2001). In our study (Mancino *et al*., 2006) 17β-estradiol induced a significant increase in the protein expression of all the three VEGF receptors with a prominent effect on VEGFR-2 (> 5-fold increase), which is a preferred receptor for both VEGF-A and VEGF-C. The effect of 17β-estradiol was mediated by either ER and by IGF1-R since it was inhibited by two specific receptor antagonists (Ici 182,780 and áIR3, respectively). Since the specificity of αIR3 for IGF1 and of Ici182,780 for ER is absolute our findings indicate a sort of interplay between ER and IGF1-R in mediating the effect of 17β-estradiol on the induction of VEGFs and related receptors. This is consistent with the proliferative effect of 17β-estradiol on HuH-28 cell proliferation which also require intact ER and IGF1-R. The VEGF induced by 17β-estradiol in HuH-28 cells is secreted in the supernatant allowing the supposition that, through paracrine mechanisms, this growth factor may participate in the proliferative effects of 17β-estradiol. On the other hand, VEGF per se plays a major role in modulating the proliferation of normal cholangiocytes as recently demonstrated in experimental studies (Gaudio *et al*., 2006).

The stimulatory effect of 17β-estradiol on the protein expression of VEGF and related receptors was blocked by antagonists of ER or IGF1-R, indicating an effect mediated by a coupling of ER and IGF1-R. Furthermore, 17β-estradiol increased the secretion of VEGF in the supernatant of HuH-28 cells. The proliferative effect of 17β-estradiol on HuH-28 cells was decreased by 75% by VEGF-Trap, a receptor-based VEGF inhibitor (Macino *et al*., 2006) indicating that most of the prolife-rative effect of estrogens is mediated by VEGF induction (Fig. 1). In substance, these findings indicate that VEGF plays a major role in mediating the proliferative effects of estrogens on human intrahepatic cholangiocarcinoma and that strategies based on the antagonism of ER and/or VEGF could help in delaying the progression of this cancer.

CONCLUSION

In conclusion, recent studies support a relevant role of estrogens as modulators of neoplastic cholangiocyte proliferation (Alvaro *et al*., 2006; Mancino *et al*., 2006). In our hypothesis, estrogens act in concert with growth factors (IGF1, VEGF) in sustaining the cholangiocyte proliferative machinery and in depressing apoptosis. This may have a number of clinical implications since pharmacological strategies aimed to inhibit estrogens binding to their receptors, to decrease their synthesis (by using aromatase inhibitors) and, finally, to down-regulate ER protein levels (by using pure anti-estrogens such fulvestant), should be considered.

REFERENCES

Adesanya, O.O., J. Zhou, C. Samathanam, L. Powell-Braxton and C.A. Bondy, 1999. Insulin-like growth factor 1 is required for G2 progression in the estradiol-induced mitotic cycle. Proc. Natl. Acad. Sci. USA., 96: 3287-3291.

Alvaro, D., G. Alpini, P. Onori, A. Franchitto, S. Glaser, G. LeSage, A. Gigliozzi, A.F. Attili and E. Gaudio, 2002. Effect of ovariectomy on the proliferative capacity of intrahepatic biliary epithelium. Gastroenterology, 123: 336-344.

Alvaro, D., G. Alpini, P. Onori, L. Perego, G. Svegliati Baroni, A. Franchitto, L. Baiocchi, S.S. Glaser, G. Le Sage, F. Folli and E. Gaudio, 2000. Estrogens stimulate proliferation of intrahepatic biliary epithelium in rats. Gastroenterology, 119: 1681-1691.

Alvaro, D., B. Barbaro, A. Franchitto, P. Onori, S. Glaser, G. Alpini, H. Francis, L. Marucci, P. Sterpetti, S. Ginanni-Corradini, A. Onetti Muda, D.E. Dostal, A. De Santis, A.F. Attili, A. Benedetti, E. Gaudio, 2006. Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. Am. J. Pathol., 169: 877-88.

Alvaro, D., V. Drudi Metalli, G. Alpini, P. Onori, A. Franchitto, B. Barbaro, S.S. Glaser, H. Francis, A. Cantafora, I. Blotta, A.F. Attili and E. Gaudio, 2005. The intrahepatic biliary epithelium is a target of the growth hormone/insulin like growth factor 1 axis. J. Hepatol., 43: 875-83.

Alvaro, D., P. Invernizzi, P. Onori, A. Franchitto, A. De Santis, A. Crosignani, R. Sferra, S. Ginanni-Corradini, M.G. Mancino, M. Maggioni, A.F. Attili, M. Podda and E. Gaudio, 2004. Estrogen receptors in cholangiocytes and the progression of primary biliary cirrhosis. J. Hepatol., 41: 905-912.

Alvaro, D., P. Onori, V. Drudi Metalli, G. Svegliati-Baroni, F. Folli, A. Franchitto, G. Alpini, M.G. Mancino, A.F. Attili and E. Gaudio, 2002. Intracellular pathways mediating estrogen-induced cholangiocyte proliferation in the rat. Hepatology, 36: 297-304.

Bardin, A., N. Boulle, G. Lazennec, F. Vignon and P. Pujol, 2004. Loss of ERbeta expression as a common step in estrogen-dependent tumor progression. Endocr. Relat Cancer, 11: 537-551.

Barkhem, T., B. Carlsson, Y. Nilsson, E. Enmark, J. Gustafsson and response of estrogen receptor alpha and beta to partial estrogen agonists/antagonists. Mol. Pharmacol., 54: 105-112.

Blum, A. and R.O. 1998. Cannon, Effects of oestrogens and selective oestrogen receptor modulators on serum lipoproteins and vascular function. Curr. Opin. Lipidol., 9: 575-586.

Brueggemeier, R.W., E.S. Diaz-Cruz, 2006. Relationship between aromatase and cyclooxygenases in breast cancer: potential for new therapeutic approaches. Minerva Endocrinol., 31: 13-26.

Cardona-Gomez, G.P., P. Mendez, L.L. DonCarlos, I. Azcoitia and L.M. Garcia-Segura, 2001. Interactions of estrogens and insulin-like growth factor-I in the brain: Implications for neuroprotection. Brain Res. Rev., 37: 320-334.

Cordera, F. and V.C. Jordan, 2006. Steroid receptors and their role in the biology and control of breast cancer growth. Semin Oncol., 33: 631-641.

Diel, P., K. Smolnikar and H. Michna, 1999. The pure antiestrogen ICI 182780 is more effective in the induction of apoptosis and down regulation of BCL-2 than tamoxifen in MCF-7 cells. Breast Cancer Res. Treat., 58: 87-97.

Diel, P., 2002. Tissue-specific estrogenic response and molecular mechanisms. Toxicol. Lett., 28: 217-224.

Eagon, P.K., M.S. Elm, M.J. Epley, H. Shinozuka and K.N. Rao, 1996. Sex steroid metabolism and receptor status in hepatic hyperplasia and cancer in rats. Gastroenterology, 110: 1199-1207.

Eagon, P.K., L.E. Porter, A. Francavilla, A. DiLeo and D.H. Van Thiel, 1985. Estrogen and androgen receptors in liver: their role in liver disease and regeneration. Semin Liver Dis., 5: 59-69.

Fava, G., M. Marzioni, A. Benedetti, A. Glaser, S. Demorrow, H. Francis and G. Alpini, 2006. Molecular pathology of biliary tract cancers. Cancer Letters, [Epub ahead of print].

Ferrara, N. and R.S. Kerbel, 2005. Angiogenesis as a therapeutic target. Nature, 15: 967-974.

Fisher, B., N. Gunduz, E.A.S. Saffer and S. Zheng, 1984. Relation of estrogen and its receptor to rat liver growth and regeneration. Cancer Res., 44: 2410-2415.

Francavilla, A., L. Polimeno, A. DiLeo, M. Barone, P. Ove, M. Coetzee, P.K. Eagon, L. Makowka, G. Ambrosino, V. Mazzaferro and T.E. Starzl, 1989. The effect of estrogen and tamoxifen on hepatocyte proliferation *in vivo* and *in vitro*. Hepatology, 9: 614-620.

Gaudio, E., B. Barbaro, D. Alvaro, S. Glaser, H. Francis, A. Franchitto, P. Onori, Y. Ueno, M. Marzioni, G. Fava, J. Venter, R. Reichenbach, R. Summers and G. Alpini, 2006. Administration of r-VEGF-A prevents hepatic artery ligation-induced bile duct damage in bile duct ligated rats. Am. J. Physiol. Gastrointest Liver Physiol., 291: G307-317.

Gaudio, E., B. Barbaro, D. Alvaro, S. Glaser, H. Francis, Y. Ueno, C.J. Meininger, A. Franchitto, P. Onori, M. Marzioni, S. Taffetani, G. Fava, G. Stoica, J. Venter, R. Reichenbach, S. De Morrow, R. Summers, G. Alpini, 2006. Vascular endothelial growth factor stimulates rat cholangiocyte proliferation via an autocrine mechanism. Gastroenterology, 130: 1270-1282.

Gigliozzi, A., G. Alpini, G. Svegliati Baroni, L. Marucci, V. Drudi Metalli, S.S. Glaser, H. Francis, M.G. Mancino, Y. Ueno, B. Barbaro, A. Benedetti, A.F. Attili and D. Alvaro, 2004. Nerve Growth Factor Modulates the Proliferative Capacity of the Intrahepatic Biliary Epithelium in Experimental Cholestasis. Gastroenterology, 127: 1198-1209.

Girault, I., C. Andrieu, S. Tozlu, F. Spyratos, I. Bieche and Lidereau, 2004. Altered expression pattern of alternatively spliced estrogen receptor beta transcripts in breast carcinoma. Cancer Lett., 215: 101-112.

Hermenegildo, C., P.J. Oviedo, A. Cano, 2006. Cyclooxygenases regulation by estradiol on endothelium. Curr. Pharm. Des., 12: 205-215.

Hyder, S.M., 2002. The role of steroid hormones on the regulation of vascular endothelial growth factor. Am. J. Pathol., 161: 345-6.

Kahlert, S., S. Nuedling, M. van Eickels, H. Vetter and R. Meyer, 2000. Estrogen Receptor a Rapidly Activates the IGF-1 Receptor Pathway. J. Biol. Chem., 75: 18447-18453.

Kassem, M., R. Okazaki, S.A. Harris, T.C. Spelsberg, C.A. Conover and B.L. Riggs, 1998. Estrogen effects on insulin-like growth factor gene expression in a human osteoblastic cell line with high levels of estrogen receptor. Calcif Tissue Int., 62: 60-66.

Kuiper, G.G., E. Enmark, M. Pelto-Huikko, S. Nilsson, J.A. Gustafsson, 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. USA., 93: 5925-5930.

Kuiper, G.J.M., P.J. Shughrue, I. Merchenthaler and J.A. Gustafsson, 1998. The estrogen receptor beta subtype: A novel mediator of estrogen action in neuroendocrine systems. Front Neuroendocrinol., 19: 253-286.

Lau, K.M., M. LaSpina, J. Long and S.M. Ho, 2000. Expression of Estrogen Receptor (ER)-alpha and ER-beta in normal and malignant prostatic epithelial cells: Regulation by methylation and involvement in growth regulation. Cancer Res., 60: 3175-3182.

Lazaridis, K.N. and G.J. Gores, 2005. Cholangiocarcinoma. Gastroenterology, 128: 1655-67.

Lazaridis, K.N. and G.J. Gores, 2006. Primary sclerosing cholangitis and cholangiocarcinoma. Semin Liver Dis., 26: 42-51.

Malhi, H. and G.J. Gores, 2006. Review article: the modern diagnosis and therapy of cholangiocarcinoma. Aliment Pharmacol. Ther., 23: 1287-1296.

Mancino, A., M.G. Mancino, A. Torrice, G. Alpini, I. Stammegna, M.C. Bragazzi, H. Francis, S. Glaser, A.F. Attili and D. Alvaro, 2006. Estrogens stimulates the proliferation of human cholangiocarcinoma by inducing the expression and secretion of VEGF. Hepatology, 44: Abstract 540.

Migliaccio, A., G. Castoria, A. Di Domenico, A. de Falco, A. Bilancio, M. Lombardi, D. Bottero, L. Varricchio, M. Nanayakkara, A. Rotondi and F. Auricchio, 2002. Sex steroid hormones act as growth factors. J. Steroid Biochem. Mol. Biol., 83: 31-35.

Mosselman, S., J. Polman, R. Dijkema, 1996. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett., 392: 49-53.

Nakamura, J., Q. Lu, G. Aberdeen, E. Albrecht and A. Brodie, 1999. The effect of estrogen on aromatase and vascular endothelial growth factor messenger ribonucleic acid in the normal nonhuman primate mammary gland. J. Clin. Endocrinol. Metab., 84: 1432-1437.

Nash, J.W., C. Morrison and W.L. Frankel, 2003. The utility of estrogen receptor and progesterone receptor immunohistochemistry in the distinction of metastatic breast carcinoma from other tumors in the liver. Arch. Pathol. Lab. Med., 127: 1591-1595.

Ogasawara, S., H. Yano, K. Higaki, A. Takayama, J. Akiba, K. Shiota and M. Kojiro, 2001. Expression of angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in human biliary tract carcinoma cell lines. Hepatol. Res., 20: 97-113.

Paige, L., D. Christensen, H. Grøn, J.D. Norris, E.B. Gottlin, K. Padilla, C.Y. Chang, L.M. Ballas, P.T. Hamilton, McDonnell and D.M. Fowlkes, 1999. Estrogen receptor modulators each induce distinct conformational changes in ER alpha and beta. Proc. Natl. Acad. Sci. USA., 96: 3999-4004.

Park, B.K., Y.H. Paik, J.Y. Park, K.H. Park, S. Bang, S.W. Park, J.B. Chung, Y.N. Park and S.Y. Song, 2006. The clinicopathologic significance of the expression of vascular endothelial growth factor-C in intrahepatic cholangiocarcinoma. Am. J. Clin. Oncol., 29: 138-142.

Ruohola, J.K., E.M. Valve, M.J. Karkkainen, V. Joukov, K. Alitalo and P.L. Harkonen, 1999. Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. Mol. Cell. Endocrinol., 149: 29-40.

Sampson, L.K., S.M. Vickers, W. Ying and J.O. Phillips, 1997. Tamoxifen-mediated growth inhibition of human cholangiocarcinoma. Cancer Res., 57: 1743-1749.

Stephen, R.L., L.E. Shaw, C. Larsen, D. Corcoran and P.D. Darbre, 2001. Insulin-like growth factor receptor levels are regulated by cell density and by long term estrogen deprivation in MCF7 human breast cancer cells. J. Biol. Chem., 276: 40080-40086.

Surmacz, E., 2003. Growth factor receptors as therapeutic targets: strategies to inhibit the insulin-like growth factor I receptor. Oncogene, 2: 6589-6597.

Wakeling, A.E., M. Dukes and J. Bowler, 1991. A potent specific pure antiestrogen with clinical potential. Cancer Res., 51: 3867-3873.

Wimalasena, J., D. Meehan, R. Dostal, J.S. Foster, M. Cameron and M. Smith, 1993. Growth factors interact with estradiol and gonadotropins in the regulation of ovarian cancer cell growth and growth factor receptors. Oncol. Res., 5: 325-337.

Zhou, J., K. Anderson, M. Bievre, S. Ng and C.A. Bondy, 2001. Primate mammary gland insulin-like growth factor system: Cellular localization and regulation by sex steroids. J. Invest. Med., 49: 47-55.