IN VITRO IDENTIFICATION OF HDL RECEPTOR, SR-B1 REGULATOR FROM SELECTED NATURAL PRODUCTS

by

KARTHIYAYINI D/O NADARASAN

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Karthiyayini D/O Nadarasan

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ANOVA	Analysis of variance
PCR	Polymerase Chain Reaction
Ct	Threshold cycle
dH_2O	Deionised water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
MeOH	Methanol
LB	Luria-Bertani
NMR	Nuclear magnetic resonance
bp	Base pair
mg	Milligram
ml	Millilitre
ng	Nanogram
nm	Nanometer (wavelength)
µM	Micromolar

µl	Microlitre
rpm	Revolutions per minute
min	Minutes
sec	Seconds
%	Percentage
v/v	Volume/volume
CO_2	Carbon dioxide
V	Volt
^oC	Degree Celsius

PENGENALPASTIAN *IN VITRO* BAGI PENGAWAL ATUR RESEPTOR HDL, SR-B1 DARIPADA PRODUK HASILAN SEMULA JADI TERPILIH

ABSTRAK

Aterosklerosis adalah keadaan di mana dinding arteri menebal akibat daripada pengumpulan dan pengekalan bahan-bahan lemak kerana ketiadaan fungsi penyingkiran lemak dan kolesterol oleh lipoprotein berketumpatan tinggi. SR-B1 adalah reseptor permukaan sel yang memainkan peranan penting dalam metabolisme kolesterol melalui laluan pengangkutan kolesterol berbalik (PKB). Esei gen pelapor telah dihasilkan dengan menggunakan rantau penggalak gen SR-B1 sebagai sasaran gen untuk menguji tindakbalas daripada produk semula jadi. Sejumlah 16 ekstrak tumbuhan dan 11 ekstrak marin telah disaring menggunakan asai tersebut. Dua ekstrak , iaitu *Andrographis paniculata* (hempedu bumi) dan *Trochus niloticus* (siput cengkerang besar) menunjukkan aktiviti positif dalam penyaringan awal. Experimen seterusnya tidak dapat diteruskan untuk *Trochus niloticus* disebabkan sampel yang terhad dan variasi antara sampel yang dikumpulkan. Oleh sebab itu, kerja seterusnya hanya diteruskan untuk *Andrographis paniculata*. Empat kompoun telah dihasilkan daripada ekstrak *A.paniculata* dan disaring menggunakan asai tersebut. Menggunakan kaedah transfeksi sementara ke atas sel, aktiviti kompoun-kompoun dalam meningkatkan aktiviti rantau penggalak SR-B1 telah dinilai. Kompoun yang menghasilkan keputusan positif telah dikenal pasti sebagai andrographolide berdasarkan spektroskopi NMR dan analisis jisim. Teknik semi- kuantitatif PCR dan analisis immunohistokimia telah digunakan untuk menentukan kesan andrographolide kepada reseptor SR-B1 dalam sel-sel HepG2. Penemuan ini telah disahkan lagi dengan melakukan asai Dil-HDL. Hasil daripada penyaringan awal

menunjukkan bahawa andrographolide merupakan pengantara transaktivasi rantau penggalak SR-B1 secara berkadar dos. Analisis immunohistokimia dan kajian gen membuktikan bahawa reseptor SR-B1 dikawal-selia oleh andrographolide. Asai Dil-HDL mensahkan lagi penghabisan HDL kolesterol dalam sel-sel HepG2 yang dijalankan oleh SR-B1 reseptor yang diaktifkan akibat rawatan andrographolide. Kesimpulannya, kajian ini menunjukkan bahawa andrographolide sebagai pengawal selia transkripsi SR-B1 dalam sel HepG2 dan mempunyai aktiviti anti-aterosklerosis.

IN VITRO IDENTIFICATION OF HDL RECEPTOR, SR-B1 REGULATOR FROM SELECTED NATURAL PRODUCTS

ABSTRACT

Atherosclerosis is a condition where the artery wall thickens as a result of the accumulation and retention of fatty materials due to an absent of adequate removal of fats and cholesterols by functional high density lipoprotein. SR-B1 is a cell surface receptor which plays a crucial role in cholesterol metabolism via reverse cholesterol transport (RCT) pathway. A reporter gene based assay was developed by implying the promoter region of SR-B1 gene as target gene to test extracts from natural products for the anti-atherosclerotic properties. Total of 16 plant extracts and 11 marine extracts were screened using the developed assay. Two extracts, namely *Andrographis paniculata* (Hempedu bumi) and *Trochus niloticus* (Giant top shell snail) showed an distinguished activity in preliminary screening. The subsequent work was unable to proceed for *Trochus niloticus* due to insufficient samples and also the samples variation between collected batches. Therefore, works were only carried out using *Andrographis paniculata* due to its samples availability. Four compounds were isolated from *A.paniculata* and subjected to screening. Utilising transient transfection approach, the potent activity of the compounds in elevating SR-B1 promoter activity was evaluated. The positive hit compound was identified as andrographolide based on NMR and mass spectroscopic analysis. Subsequently, semi-quantitative real time PCR and immunohistochemistry was employed to observe the effect of andrographolide on SR-B1 mRNA expression and its protein content in HepG2 cells respectively. These findings were further validated by performing Diotadecylindocarbocyanine-High Density Lipoprotein (Dil-HDL)

uptake assay. The preliminary screening performed using transient transfection approach demonstrated that andrographolide mediated the trans-activation of SR-B1 promoter, thereby increased the transcriptional activity of SR-B1 in dose dependent manner. Subsequently, immunohistochemistry and mRNA expression study illustrated the mRNA and the protein expressions of the SR-B1 was up-regulated by andrographolide. Similarly, the Dil-HDL assay further confirmed the HDL cholesterol efflux was significantly improved in andrographolide-treated HepG2 cells. Conclusively, we have demonstrated andrographolide as a potential transcriptional up-regulator of SR-B1 in HepG2 cells and possesses anti-atherosclerotic activity.

CHAPTER 1

INTRODUCTION

1.1 Research background

Atherosclerosis is the condition in which an artery wall thickens as a result of the accumulation and retention of fatty materials. This condition occurs due to absent of adequate removal of fats and cholesterols from blood circulation and wall of blood vessels (Acton et al.,1999). Atherosclerosis has become one of the leading killer diseases in most of the developing countries including Malaysia (Bao et al., 2009). Genetic factors such as diabetics and hypercholesterolemia as well as the environmental factors such as smoking and unhealthy diet highly correlated to the incidence of atherosclerosis (Sainani et al., 2008; Jamkhande et al., 2013). Although there are many advances in research in related areas, atherosclerosis still remains a major medical problem which resulted in disastrous clinical consequences such as heart attack.

Presently, there are many lipid-lowering drugs available in current market which are able to regulate the level of cholesterol content in the body. For example, statins, fibrates and niacins are well known lipid lowering drugs extensively used to treat various lipid-related diseases (Drexel, 2009; Pal, 2009). Despite various cholesterols lowering therapies are widely available in current market, the scenario of cardiovascular disease especially atherosclerosis remains as leading cause of death globally. If this scenario continuous, it may leads to more severe consequences which potently claim extremely huge number of lives. Therefore, this critical

situation has advocated an urgent need to discover an anti-atherosclerotic agent with robust effect to overcome the atherosclerotic event effectively. Additionally, invention of new therapeutics by targeting the clearance of existing or deposited cholesterols in body may eventually lead a way to healthier life and reduce the risk of coronary heart disease (CHD).

Reverse cholesterol transport (RCT) is a protective mechanism which plays a very important role in cholesterol metabolism and potently resist the incidence of atherosclerosis. This pathway mainly emphasises on disposal of excessive free cholesterol in blood stream with the presence of functional high density lipoprotein (HDL) molecules (Fielding and Fielding, 1995). Interestingly, the active HDL uptake and the removal of cholesterols into liver cells is facilitated through the activation of a special receptor, scavenger receptor class B type 1 (SR-B1) (Chao et al., 2010). SR-B1 receptor binds to HDL with high affinity and mediates the uptake of cholesteryl esters from HDL into the liver for disposal (Yumiko et al, 2005, Bao et al, 2009). Focusing new strategies by targeting SR-B1 receptors may open a new therapeutic invention for the treatment of atherosclerotic cardiovascular disease. Therefore, in our study we focused on the regulation of SR-B1 gene to identify potential ligand from natural products which able to halt the progression of atherosclerosis without serious side effects.

Plant natural products such as medicinal plants or phytomedicines are widely used to treat various diseases including cardiovascular disease (CVD). Conventionally, the parts of the plants such as roots, flowers and fruits are used to treat many disorders (Tang and Halliwell, 2010). Past studies have shown that

research communities have documented many medicinal properties of plants in the ethnopharmacology field, including as anti-atherosclerotic agents (Kris-Etherton et al., 2002; Obute and Adubor, 2007). Besides that, there is a growing interest in marine natural products due to its incredible diversity and greatly rich in pharmaceutically active compounds (Stankevicins et al., 2008). There is still vast percentage of marine organisms unidentified or poorly explored by the researchers due to its poor accessibility (Bugni et al., 2008**)**. Previous studies on marine organisms have proven that marine natural product exhibit a broad range of activities including cardiovascular disorders. Additionally, most of the secondary metabolites produced by marine organisms are very exclusive with distinctive structural features compared to other natural resources (Stankevicins et al., 2008). These factors direct our interest to discover potential industrial and medical applications from plant and marine natural products for cardiovascular disease especially atherosclerosis.

1.2 Objectives of the study

As described earlier, the role of SR-B1 in mediating RCT is pivotal in reducing the progression of atherosclerosis. Therefore, the objectives of the study were

1. to construct SR-B1 promoter based luciferase screening assay

2. to screen and identify the potential candidate(s) from natural products that induce the transcriptional activity of SR-B1 promoter

3. to validate the effect of the potential candidate(s) on SR-B1 via gene and protein expression studies and lipid uptake assay.

The study is important not only for advancing our understanding of the effects of natural products in regulating the transcriptional activity of the SR-B1 promoter, but in longer term, may also possibly lead to the identification of candidates for therapeutic intervention of the lipid related diseases. This will enable specific drugs to be designed based on identified target of the diseases and thereby benefits the community especially those are suffering from various cardiovascular disorders.

CHAPTER 2

LITERATURE REVIEW

2.1 Atherosclerosis

Atherosclerosis is a type of cardiovascular disorder (CVD) categorised as one of the non-communicable and chronic inflammatory diseases. The accumulation and retention of fatty materials results in formation of thrombosis and hemorrhage in arterial wall lead to atherosclerosis (Figure 2.1). As the percentage of blockage increased in artery, this condition ended in serious cardiovascular complications such as heart attack, stroke and also death due to insufficient supply of oxygen-rich blood to organs and other parts of body (Castelli et al., 1986; Robert, 2005). Atherosclerosis has become one of the leading killer diseases in most of the developed and developing countries including Malaysia (Margaret, 2010; Bao et al, 2009). World Health Organisation (WHO) has estimated 17.3 million people died from CVDs in 2008 and over 80 % of CVD deaths worldwide take place in low- and middle-income countries. According to WHO forecast, more than 23 million people will die annually from CVDs by 2030 (http://www.who.int/cardiovascular_ diseases/en/). Apart from that, the National Health and Morbidity Survey (NHMS) conducted by the Institute of Public Health in 2011 reported that 35.1% that is about 6.2 million of adults who are 18 years and above have hypercholesterolemia in Malaysia (National Health and Morbidity Survey, 2011).

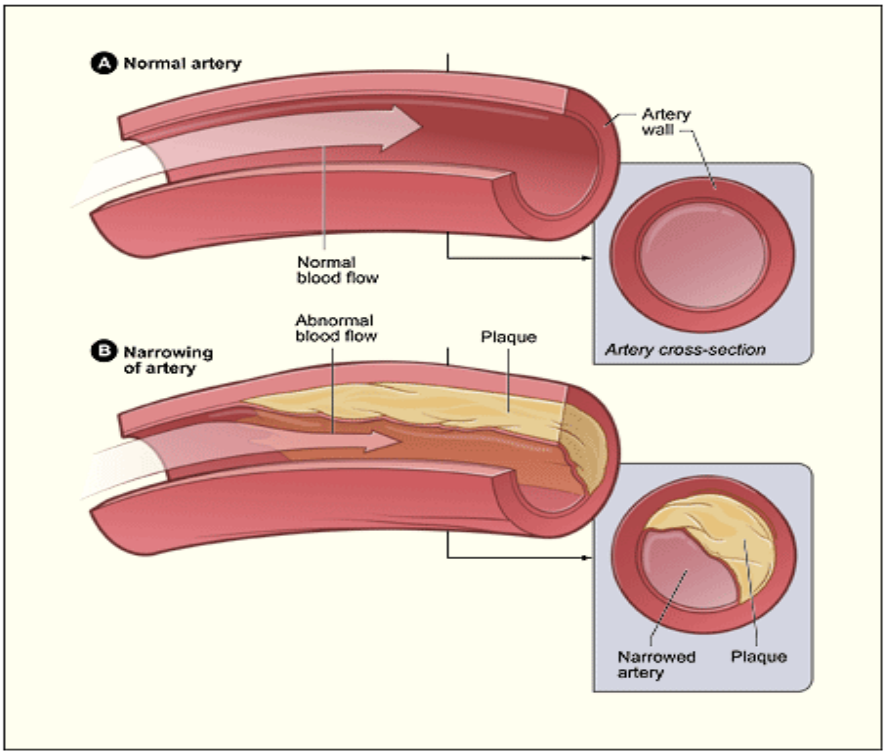

Figure 2.1 Atherosclerosis (A) shows a normal artery with normal blood flow (B) shows an artery with developed atherosclerotic plaque (Source: http://www.medicalnewstoday.com/articles/247837.php)

Cholesterol is a waxy, fat-like substance found in the cell membrane of all body tissues. It is very important to our body as it involves in many biochemical processes such as production of steroid hormones, vitamin D and some essential substances for food digestion. Cholesterol is insoluble in blood and transported in the circulatory system via carriers called lipoprotein. Lipoprotein can be classified into 2 main groups, namely low-density lipoprotein (LDL) and high-density lipoprotein (HDL) which involve in cholesterol metabolism by carrying the lipid components from and to the liver. The excessive LDL cholesterol presents in the blood stream initiates the built-up of atherosclerotic plaques at the inner wall of arteries, thereby causing narrowing of arterial wall and leading to progression of atherosclerosis (Pal, 2009). Conversely, a high level of HDL is beneficial as it prevents the occurrence of atherosclerosis and also helps in regression of atherosclerotic condition.

Immune and inflammatory mediators are the major factors for the initiation and development of atherosclerosis. Various reports described atherosclerosis as an inflammatory event due to its occurrence highly associated to numerous cellular and molecular pathways. The process of atherogenesis has explained in two different theories. The first theory is 'response to injury' which explains the injuries occurred in the endothelial lining promote the deposition of LDL cholesterol in the intimal space and undergo modification. The modified LDL molecule is recruits by monocyte-derived macrophages and re-localised become foam cells (Ross et al., 1977; Ross, 1993). The second hypothesis is 'response to retention', which clarify the removal of modified LDL components by macrophages from the intimal space via scavenger receptors and become foamy (Williams and Tabas, 1995).

Briefly, the accumulated LDL cholesterols in the intimal space of endothelial cells undergo modification by oxidation. As the result of oxidation process by some LDL-oxidative enzymes such as lipoxygenases, myeloperoxidase and NADPH oxidases, the LDL converted into oxidised form (Li and Glass, 2002). Subsequently, the expression of adhesion molecules, such as intracellular adhesion molecule, vascular cell adhesion molecule, E-selectin and P-selectin are enhanced by the activated endothelial cells. Adhesion molecules play key roles in the recruitment of monocytes in the arterial wall. P-selectin binds to P-selectin glycoprotein ligand-1 presents on monocytes which allows the capturing, rolling and activation of monocytes (Elstad et al., 1995; Weyrich et al., 1995). According to Chandak et al. (2011), P-selectin and E-selectin play similar role in atherosclerosis progression. The recruitment of monocyte and its firm adhesion to the endothelial surface are facilitate by vascular cell adhesion molecule. Besides, the adhesion, spreading and migration of monocyte into the sub-endothelial space are mediate by intracellular adhesion molecule (Chandak et al., 2011). Endothelial cells and smooth muscle cells secrete macrophage colony-stimulating factor, which differentiates the monocytes into macrophages in the endothelial space. Upon conversion, the endothelial cells induce the expression of microphage-chemoattractant protein-1; produced by activated microphages, in the presence of oxidised LDL. Consequently, the oxidised LDL undergo lysosomal degradation producing free cholesterols which result in foamy macrophages. The accumulation of lipid-laden microphages (foam cells) in the arterial wall is the major trait of atherosclerosis (Li and Glass, 2002).

2.1.1 Atherosclerotic risk factors

Progression of atherosclerotic plaque is a complex process, influenced by various genetic and environmental risk factors. In human, genetic enzymatic dysfunction, dietary deficiency as well as other environmental risk factors such as smoking, high fat diet and infectious agents promote the development of atherosclerotic lesions in humans (Sainani et al., 2008).

Elevated cholesterol level is an independent pioneer trait greatly contributes to the progression and incidences of atherosclerosis (Sarah and Bruce, 2009). Cholesterol is an important substance to our body for the production of hormones and bile acids. However, the poor management of cholesterol due to impaired metabolic systems or pathways leads to hypercholesterolemia. This condition exaggerates the formation of plaques due to the deposition of excessive free and oxidised cholesterols along blood vessels (Yun et al., 2005).

The high sugar level in diabetes patients, especially type 2 diabetes patients often leads to atherosclerotic development. This complication resulted in more than 80 % in overall diabetic death (Sainani et al., 2008). The enzymatic dysfunction in diabetes patients causes elevated level of LDL and triglycerides level, lessen the amount of HDL in plasma as well as promotes coronary calcification which are directly correlated to the risk of CHD (Bradford and Thomas, 2002; Sarah and Bruce, 2009). Consequently, diabetes represents as a major threat of atherosclerotic event globally (Yun et al., 2005).

Smoking is one of the environmental jeopardize factor which greatly aggravates and speeds up the growth of atherosclerotic lesions in the blood vessels. Cigarette smoke boost the arterial wall stiffness by shooting up the fibrinogen level, hematocrit and platelet aggregation, thus eventually decreases HDL level (Spence, 2007; Keaney, 2000; Sarah and Bruce, 2009). According to Surgeon General update report, smoking enhances atherosclerotic disease by more than 50 % and increases the occurrences of CHD by two fold high (Keaney, 2000). The dissimilarities in genetic variants between individuals also related to the high risk of atherosclerosis in smokers compared to non-smokers (Bradford and Thomas, 2002).

2.2 Reverse Cholesterol Transport

Reverse cholesterol transport (RCT) is a principal protective system that plays a pivotal role in reducing the progression of atherosclerosis. It is referred to the opposing movement of cholesterol from peripheral region to the liver (Figure 2.2) and steroidogenic organs (Schoonjans et al., 2002).

The primary step in RCT is the removal of cholesterols from lipid laden cells found in the arterial wall. High density lipoprotein (HDL) plays a crucial role in the clearance of excessive cholesterols from extracellular matrixes. Approximately 70 % of HDL comprised of apoA-1 triggers the cholesterol efflux in the cells via RCT. The interaction of apoA-1 with ATP binding membrane cassette transport protein A1 (ABCA1) present on the cell surface of lipid laden cells initiates the lipid mobilization (Wang et al., 2007a). Upon activation, the functional HDL recruits unesterified and free cholesterols from extra hepatic tissues. The recruited lipid substances will undergo esterification by lecithin-cholesterol acyltranferase (LCAT) on HDL forming cholesteryl esters (CE), eventually resulting in nascent HDL due to the increased amount of CE in this lipoprotein fraction. These larger HDL molecules able to interact with ATP binding membrane cassette transport protein G1 (ABCG1) and promote more lipid extraction from lipid-rich peripheral tissues (Wang et al., 2007b).

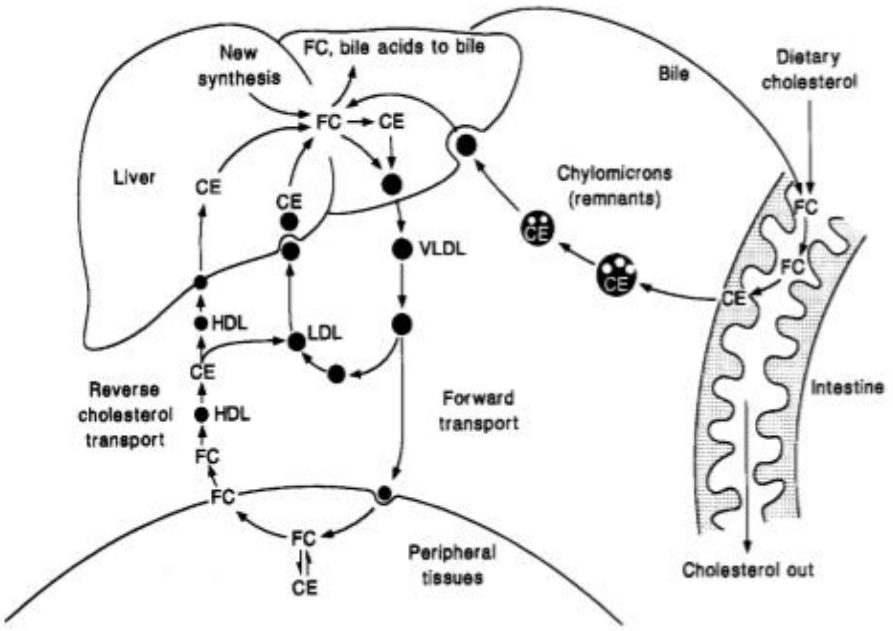

Figure 2.2 Schematic diagram of reverse cholesterol transport shows major cholesterol pathways between liver and peripheral tissues. Arrows indicate the direction of net transport. FC- Free cholesterol, CE- Cholesteryl ester, HDL- High density lipoprotein, LVL- Low density lipoprotein, VLDL- Very low density lipoprotein (Source: Fielding and Fielding, 1995).

The next essential step of RCT is the delivery of the esterified cholesterols to the liver and other steroidogenic tissues. The transfer across membrane is highly mediated by cholesterol transporter such as scavenger receptor class B type 1 **(**SR-B1) receptor. The SR-B1 receptor located at cell surface membrane of hepatocytes facilitates the transfer of CE from HDL into liver. The CEs are transferred from HDL to apolipoprotein (apo) B-containing lipoproteins by cholesteryl ester transfer protein (CETP) prior to be taken up by RCT terminal via SR-B1. In this process, HDL is not been degraded or discarded through enzymatic reactions or endocytosis. In fact, the HDL molecule acts as shuttle which delivers the CE to the liver via SR-B1 receptor and subsequently returns back to peripheral tissues to transfer more free cholesterol to the liver for disposal (Kinoshita et al., 2004; Ono, 2012).

Excretion of cholesterol from body is the last step in the RCT route. In liver, the transported CE will be converted into bile acid and excreted out from the body. The bile reabsorbed in the intestine and secreted along with dietary cholesterol into the intestinal lymph, and subsequently reappears in the plasma as chylomicrons. The clearance of these chylomicron remnants which conserved most of their cholesterol content is rapidly done by the liver. Alternatively, the transported CE is used for steroid hormones synthesis in steroidogenic cells (Kocher et al., 2008; Rigotti et al., 1996).

2.2.1 High Density Lipoprotein

High-density lipoprotein (HDL) has been characterised as heterogeneous molecules and metabolically active group of lipoproteins that exhibits beneficial influence on atherosclerosis and subsequently cardiovascular diseases (Davidson and Toth, 2007; Linsel and Tall, 2005). The protection of atherosclerosis event by functional HDL is through its action in reverse cholesterol transport (Lewis and Rader, 2005; Toth, 2003).

Numerous epidemiological studies have revealed that the level of HDL in blood inversely proportional to the incidence of atherosclerosis. A six years PROCAM follow-up study demonstrated the incidence of CHD reduces as the quantity of HDL-C increases (Assmann et al., 1996) while Framingham Heart Study illustrated the risk of CHD lessen with increased level of HDL-C regardless to the amount of LDL (Gordon et al., 1977). In addition, many observational studies performed globally have confirmed the anti-atherogenic properties of HDL in people irrespective of sex, race, or ethnicity (Castelli et al., 1986; Sharrett et al., 2001). HDL possesses a range of pleiotropic effects such as anti-inflammatory (Barter et al., 2002; Cockerill et al., 2001), anti-oxidative (Navab et al., 2000), anti-apoptotic (Kimura et al., 2001; Nofer et al., 2001), anti-thrombotic (Epand et al., 1994; Viswambharan et al., 2004), vasodilatory (Spieker et al., 2002) and cholesterol mobilization properties (Zhang et al., 2003), all of which underlie their potential athero-protective nature.

HDL metabolism is a multifaceted mechanism involving HDL synthesis, intravascular remodeling and catabolism (Tall, 1990). The concept of reverse cholesterol transport closely associates to HDL metabolism clearly explains the task of HDL as athero-protective marker and its ability to repress atherosclerosis. HDL modulates the lipid homeostasis by mediating selective lipid uptake. Studies carried out with labelled HDL clearly explain the athero-protective role of HDL in RCT (Pieters et al., 1994).

2.2.2 Current lipid management therapy on HDL cholesterol

The condition of dyslipidemia is the key factor resulted in cardiovascular disorder especially atherosclerosis in many patients. Besides practicing lifestyle changes such as weight reduction, exercise and smoking cessation; advanced therapeutic treatments are required to halt the extensive rise of atherosclerotic event.

Many years ago, the management of atherosclerosis due to dyslipidemia started with statin-therapy. Statins have only shown to halt the progression of atherosclerosis about 16% (Brewer, 2003). Statins acts by stimulating ATP-binding cassette transporter A1 (ABCA1) expression in the hepatic tissues through a liver-specific promoter element (LPE). This lipid lowering drug reduces the content of apolipoprotein (apo) B lipoproteins in the blood stream and eventually decrease the rate of CE transfer from HDL by CETP (Le Goff et al., 2004). Moreover, the enhancement of hepatic apoA-1 production which triggers by some statins raised the nascent HDL in the extracellular region (Schaefer et al., 1999).

In addition, niacin, a soluble B vitamin is another drug have shown to induce the HDL cholesterol levels by 20-30% in clinical trials (Birjmohun et al., 2005). Niacin engaged in HDL elevation process via three systems. First, it inhibits the hormone sensitive triglyceride lipase which suppress triacylglycerol lipolysis and minimize triglyceride level in blood (Karpe and Frayn, 2004). Secondly, niacin assists in cholesterol efflux from macrophage to HDL acceptors via ABCA1 transporters (Rubic et al., 2004). The third mechanism involve the ability of niacin to reduce the uptake of HDL particles by the disposal terminals such as liver (Jin et al., 1997; Shepherd et al., 1979).

Moreover, fibrates, PPARα agonists have shown to elevates the level of HDL by up to 10 % only (Birjmohun et al., 2005). The elevation of HDL by fibrates involved several steps. Initially, it regulates the expression of apo A-I and apo A-II. Then, ABC1 and SR-B1 are up-regulated and control the macrophage cholesterol efflux. Finally, the VLDL lipolysis raised by the induced expression of lipoprotein lipase (LPL), releasing HDL particles (Chapman, 2003; Fruchart et al., 2001).

Other than these, triazolidinediones (Raskin et al., 2001), JTT-705 (Okamoto et al., 2000) and Torcetrapib (Clark et al., 2006) have been also reported on their ability to increase the level of HDL cholesterols at lower level. The actions of currently available drugs in enhancing the HDL cholesterol are considered inadequate as there is still remain a larger burden of residual risk of CHD lacking solution around the world.

2.3 Scavenger Receptor Class B Type 1 (SR-B1)

Scavenger Receptor Class B Type 1 (SR-B1) is a 509 amino acid, ~82kDa cell surface glycoprotein belongs to CD36 family. SR-B1 contains two cytosolic regions flanking at N and C terminus. Initially, human SR-B1 (hSR-B1) identified as a protein with unknown function called CLA-1 and mapped to human chromosome-12. There is two E-box consensus sequences located on hSR-B1 promoter region, one at proximal (-160bp) and the other is at distal (-1145bp) to the transcription start site (Cao et al., 1997). SR-B1 protein embedded in the plasma membrane via two transmembrane domains. This receptor expressed abundantly in liver, adrenal gland, ovary and testis. The hSR-B1 exhibits tissue-specific pattern expression. Unlike scavenger receptor class A and C, the binding specificity of this receptor to various lipoprotein in variety of cell types and tissues is very unique (Acton et al., 1996; Cao et al., 1997; Landschulz et al., 1996).

2.3.1 SR-B1 as anti-atherosclerotic receptor

SR-B1 is a multi-ligand receptor closely associated to cholesterol homeostasis. The discovery of SR-B1 as HDL receptor has advocated the interest to investigate its potential role in HDL metabolism and the significance relevance in atherosclerosis. SR-B1 represent as an anti-atherosclerotic receptor plays an important role in RCT and therefore may eventually attenuates the incidence of CHD (Kocher et al., 2008). The selective uptake of cholesterol by HDL is merely stimulated by the expression of SR-B1 receptors and provide protection against atherosclerosis by being a key regulator in RCT (Rigotti et al., 1997). Studies have demonstrated that SR-B1 possesses athero-protective activity (Arai et al., 1999). For

example, investigation carried out using animal model showed that over expression of SR-B1 suppresses atherogenesis in LDL receptor knock-out mice and the combined inactivation of the apo-E and SR-B1 genes in mice results in dramatically enhanced aortic root atherogenesis compared to apo-E knock-out mice (Kocher et al., 2008). There is another study illustrated SR-B1's essential role in selective cholesterol transport, balancing the plasma lipoprotein and biliary cholesterol levels, HDL structure remodeling and RCT (Trigatti et al., 1999). As well, the central role of SR-B1 in hepatic selective uptake of HDL cholesterol was clearly established (Varban et al., 1998).

2.3.2 Transcriptional regulation of SR-B1 expression in liver

Numerous studies have scientifically proven the regulation of hepatic SR-B1 expression at transcriptional level by various potential molecules. A number of nuclear factors (PPAR, LXR, FXR, SF-1, LRH-1, estrogen receptors and SREBPs) and stimulation of some intracellular metabolic pathways via some potential ligands (IGF-1, glucose, leptin, inflammatory agents, vitamin A and synthetic compounds) are able to modulate the SR-B1 mRNA level. Indeed, the consensus sequences of some of these nuclear response elements motifs which are responsible for the interaction with SR-B1 have been identified in the promoter region of human and rat SR-B1 (Figure 2.3) (Rhainds and Brissette, 2004).

A few peroxisome proliferator-activated receptors (PPAR) ligands such as fatty acids, fibrates and glitazones have shown to involve in SR-B1 gene regulation. A marked increase in SR-B1 mRNA and protein expression observed in hamster fed with high contain of fatty acid diet (Spady et al., 1999). Similarly, treatment with selective PPARγ and PPARα agonists induced hepatic SR-B1 expression *in-vivo* and *in-vitro* respectively (Lopez and McLean, 2006; Malerod et al., 2003). In addition, Malerod et al., (2002) demonstrated liver X receptors (LXR) participation in controlling SR-B1 expression. LXR stimulation by oxysterols enhanced SR-B1 expression and concurrently mediates cholesterol excretion via bile synthesis. A recent study showed that the activation of farnesoid X receptors (FXR) by its appropriate agonist elevates SR-B1 expression in association with changes in hepatic regulatory factors (Zhang et al., 2010). Besides, the expression of SR-B1 was diminished in the liver of FXR deficiency mice (Lambert et al., 2003).

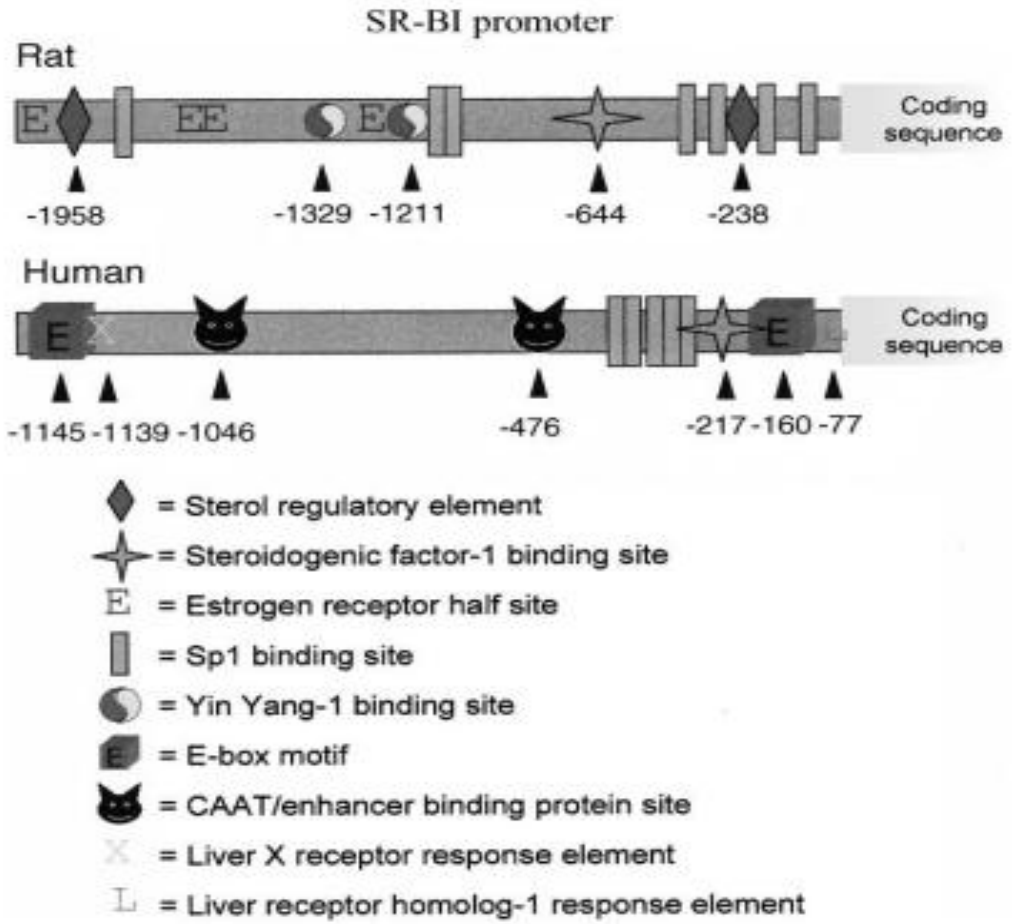

Figure 2.3 Graphical representation of important regulatory and structural elements in rat and human SR-B1 promoter region. Black triangles and numbers indicate nucleotide position relative to translation initiation codon (Source: Rhainds and Brissette, 2004).

Finally, *in-vitro* and *in-vivo* studies have exemplified the positive regulation of SR-B1 by liver receptor homolog-1 (LRH-1) (Schoonjans et al., 2002). In contrast, fibrates administration leads to a drop in SR-B1 protein level in the liver of experimental mice (Mardones et al., 2003). Other than these, estrogen treatment and sterol regulatory element binding proteins (SREBPs) are also possesses regulatory effects on hepatic SR-B1 expression (Horton et al., 2003; Lopez and McLean, 1999).

Likewise, the activation of secondary messenger pathways by some endogenous and exogenous compounds also modulate the SR-B1 expression in hepatocytes. The endogenous compounds such as insulin-like growth factor-1 (IGF-1) (Cao et al., 2004), glucose (Murao et al., 2008) and few pro-inflammatory agents showed activation of hepatic SR-B1 mRNA level. Leptin, an adipokine have shown up-regulation of SR-B1 at mRNA and protein level which initially modulating the overall energy metabolism (Lundasen et al., 2003). Interestingly, vitamin A, an exogenous compound significantly raised SR-B1 expression in the liver and subsequently an increased quantity of SR-B1 protein and decreased level of HDL cholesterol were observed in the obese phenotypes (Jeyakumar et al., 2007). Moreover, some synthetic lipid lowering compounds (Lukic et al., 2003; Mendez-Gonzalez et al., 2010) and isoflavones such as pratensein (Yang et al., 2009) have been also reported on their abilities to up-regulate SR-B1 mRNA expression and enhanced the uptake of HDL cholesterol. Recently, trichostatin A (TSA); a synthetic compound has been described on facilitating trans-activation of SR-B1 promoter activity and boost the protein expression in hepatic cell cultures (Bao et al., 2009).

2.3.3 Post-transcriptional regulation of SR-B1 expression in liver

Beside transcriptional control, the post- transcriptional regulation of SR-B1 have also been reported. The down-regulation and up-regulation of SR-B1 protein expression is facilitated by various natural products and synthetic drugs.

Particularly, some hormones and pharmacological agents potentially drive the post-transcriptional up-regulation of SR-B1. Thyroid hormones such as triiodothyronin (T_3) and tetraiodothyronine (T_4) have shown to induce the hepatic SR-B1 proteins as well as other proteins involves in cholesterol homeostasis (Johansson et al., 2005; Tancevski et al., 2010). On the other hand, insulin treatment enhances the SR-B1 protein bioavailability in the liver by promoting translocation of the protein receptor via P13K pathway. The raised expression of SR-B1 receptors on hepatic cell surfaces due to insulin, improves HDL mediated- cholesterol efflux without concomitant changes in SR-B1 gene expression (Shetty et al., 2006). Interestingly, phosphatidylethanolamine N-methyltransferase (PEMT); a liver specific enzyme shows an inverse relation of the post-transcriptional expression of SR-B1. Hence, the enzymatic dysfunction of PEMT leads to induction of SR-B1 protein expression regardless of unchanged mRNA level (Robichaud et al., 2008).

On the contrary, action of some hormones, vitamin and synthetic compounds leads to down-regulation SR-B1 protein expression in the liver without simultaneous effect on the gene expression. Treatment of 17β- estradiol on human hepatocytes and estrogen supplemented animal model demonstrated suppression of SR-B1 protein receptors (Zhang et al., 2007). In addition, another *in-vivo* and *in-vitro* studies

implied vitamin E as supplement resulted in reduction of hepatic SR-B1 protein level (Keaney et al., 1999; Witt et al., 2000). The usage of fibrates, a synthetic PPARα ligand associated with a significant drop in SR-B1 protein in the liver without any modifications of mRNA level. It was demonstrated that this drug disturbed the protein stability in the peripheral membrane and led to degradation (Lan and Silver, 2005; Mardones et al., 2003).