



Review

Effects of aldosterone on coronary function

Ludovic Benard, Paul Milliez, Marie-Lory Ambroisine, Smail Messaoudi, Jane-Lise Samuel, Claude Delcayre

INSERM U 942 and University Paris-Diderot, Hospital Lariboisiere, 41 Boulevard de la Chapelle, 75010 Paris, France

Correspondence: Claude Delcayre, e-mail: Claude.Delcayre@inserm.fr

Abstract:

Our understanding of the effects of aldosterone and its mechanisms has increased substantially in recent years, probably because of the importance of the mineralocorticoid receptor (MR) antagonists in several major cardiovascular diseases. Recent clinical studies have confirmed the benefits of MR antagonists in patients with heart failure, left ventricular dysfunction after myocardial infarction, hypertension or diabetic nephropathy. However, it would be a gross oversimplification to conclude that the role of aldosterone is unequivocally negative.

Aldosterone is synthesized in the adrenal glands and binds to specific MRs in target epithelial cells. The steroid-receptor complex penetrates the cell nucleus where it modulates gene expression and activates specific aldosterone-induced proteins that control sodium reabsorption. Recent studies have shown that aldosterone also impacts a wide range of non-epithelial tissues such as the heart and blood vessels. Remarkably, aldosterone can also be synthesized in extra-adrenal tissues and it may act in a rapid non-genomic manner. We note the existence of glucocorticoids that exhibit plasma concentrations much higher than those of aldosterone and that are structurally very similar to aldosterone. It is thus possible that glucocorticoids may bind to the aldosterone receptor in some cell types.

Diverse experimental models and several strains of transgenic mice have allowed us to better understand the effects of aldosterone on the heart. Specifically, it seems that a slight increase in cardiac aldosterone concentrations induces a decreased coronary reserve in mice by decreasing the BKCa potassium channels associated with coronary smooth muscle cells. Taken together, these experiments indicate that vascular cells are the primary targets of aldosterone in the cardiovascular system. The hormone directly affects NO and EDHF-mediated coronary relaxation. Both mechanisms may contribute to the deleterious cardiovascular effects of MR stimulation.

Key words: aldosterone, coronary function, heart failure, cardiac fibrosis, angiotensin

Abbreviations: 11-HSD2 – 11-beta-hydroxysteroid dehydrogenase type II, ACE – angiotensin converting enzyme, ACTH – adrenocorticotrophic hormone, Ang II – angiotensin II, AT1-R – angiotensin receptor type 1, BKCa channel – big conductance calcium-activated potassium channel, Cox-2 – cyclooxygenase 2, EDHF – endothelium derived hyperpolarizing factor, EGFR – endothelium growth factor receptor, GR – glucocorticoid receptor, GRE – glucocorticoid responsive element, MCP1 – monocyte chemo-attractant protein 1, MR – mineralocorticoid receptor, NO – nitric oxide, NOS – nitric oxide synthase, SGK – serum glucocorticoid responsive kinase, SMC – smooth muscle cells

Introduction

The RALES [38] and EPHESUS [37] clinical studies have demonstrated the very real benefits of MR antagonists for patients with heart failure or with left ventricular dysfunction that follows myocardial infarction. In parallel, experimental studies using very high concentrations of aldosterone have helped scientists to understand a series of related mechanisms, all

of which result in deleterious remodeling of the heart and blood vessels. However, it would be a gross oversimplification to conclude that the role of aldosterone is unequivocally negative. To better understand its mechanisms and better anticipate the effects of treatment, this article serves to highlight several points of interest regarding aldosterone and its impacts.

The classical role of aldosterone involves the regulation of the body's hydro-mineral balance as a means of ultimately controlling blood pressure. Experimental studies have demonstrated that when the level of aldosterone increases in the plasma or in target tissues, we observe a range of structural and functional changes in the heart, kidneys and blood vessels. These deleterious effects include cardiac and renal fibrosis, inflammation and vascular remodeling, and changes associated with fibrinolysis. In clinical situations including primary hyperaldosteronism, post-myocardial infarction or chronic heart failure, these types of damage are somewhat attenuated since the levels of aldosterone are to some extent controlled. These effects are therefore said to be mediated by aldosterone, since the MR antagonists spironolactone or eplerenone serve to prevent or minimize the occurrence of these effects. Note that we cannot discount the notion that potassium and hypertension may also play a key role in these deleterious changes. However, it is important to stress that these effects are due to aldosterone concentrations that are chronically elevated, and that are incompatible with the body's salt requirements [1]. In addition, a distinction must be made between inhibition of MR and the use of aldosterone antagonists. Indeed, we note the presence of plasma glucocorticoids at concentrations much higher than those of aldosterone, and the structural similarity between these two hormones and their receptors. The hormone-receptor affinities measured *in vitro* suggest the possible binding of cortisol (or corticosterone in rodents) to the aldosterone receptor. These data complicate our understanding of the mode of action of aldosterone in the cardiovascular system.

Biosynthesis of aldosterone

In the 1930s, research groups led by Kendall and Reichstein found evidence for a substance that was secreted by the cortex of the adrenal glands and that

promoted salt retention in humans. For a historical review of the discovery of aldosterone, see [45]. From the crystallization of glucocorticoids and mineralocorticoids, researchers then discovered that a certain part of the fraction extracted from the adrenals could not be crystallized. This so-called "amorphous" fraction had an important mineralocorticoid activity, albeit different from that of deoxycorticosterone or other steroids. It was not until the 1950s and the improvement of biochemical techniques that certain researchers started to show an interest in this fraction, since they were convinced of the existence of a mineralocorticoid that was different from deoxycorticosterone. This active fraction, first named electrocortin because of its properties in respect of electrolyte metabolism, was ultimately crystallized. The hormone was subsequently renamed aldosterone. Tait and colleagues confirmed that this hormone was indeed secreted by the adrenal glands. These researchers showed that the hormones extracted from cow or dog adrenal perfusates were identical. Across the decades, there has been a certain tension between those who believed that cortisol was the genuine adrenal hormone (and that aldosterone was an artifact of the synergistic action of steroids), and others who maintained that aldosterone was a hormone essential to the regulation of electrolyte metabolism.

After these discoveries, a series of experiments established that aldosterone can be synthesized from cholesterol in the zona glomerulosa of the adrenal cortex. The final biosynthesis steps involve the mitochondrial P-450 aldosterone-synthase, which catalyzes the 11 β -hydroxylation of deoxycorticosterone to form corticosterone. Through an 18-hydroxylation and 18-methyloxidation step, this ultimately yields aldosterone [3]. Aldosterone-synthase is encoded by the CYP11B2 gene and its activity is stimulated mainly by Ang II and potassium, and more weakly by ACTH and sodium. Extra-adrenal sites of aldosterone production have been also identified, including the brain [16], blood vessels [20] and heart [23, 43]. Aldosterone synthesis in these organs is generally low under resting conditions, suggesting that the secreted hormone exhibits minimal autocrine or paracrine activity. The relevance of aldosterone synthesis in heart muscle remains unclear [15], but several observations have been made in the context of pathological conditions under which the well-described stimulation of the intracardiac renin-angiotensin system may lead to increased aldosterone concentrations within the car-

diac tissue, with possible deleterious consequences. This has been observed in post-myocardial infarction scenarios in rats, where the increased Ang II and aldosterone cardiac production can play a key role in the development of cardiac fibrosis [42]. Similarly, aldosterone production is activated in humans in proportion to the severity of a failing ventricle [32]. Myocardial aldosterone and aldosterone synthase mRNA levels are elevated by 4- to 6-fold in humans with hypertrophic cardiomyopathy. We also note the increased expression of hypertrophic markers in rat cardiac myocytes and the expression of collagens and the transforming growth factor beta-1 in rat cardiac fibroblasts [46].

Vascular effects of aldosterone

The pioneering work of Selye confirmed that the administration of the mineralocorticoid deoxycorticosterone improved the survival of adrenalectomized rats. However, adverse effects (namely a cardiac necrosis) were also observed [6]. This early observation led to significant interest in the role of mineralocorticoid hormones in cardiovascular disease. Several teams have observed the induction of a peri-inflammatory phenotype in the heart tissue of uninephrectomized rats that had been treated with an aldosterone-salt complex (review in [9]). An increase in the concentrations of inflammation markers such as Cox-2 and MCP-1 can be seen from the first week onwards. Scientists have concluded that the proliferation of inflammatory cells around the coronary arteries may be one of the earliest events leading to fibrosis [40]. The causal role of oxidative stress is suggested by the fact that spironolactone and antioxidants prevent these changes from occurring independently in the coronary [44] or peripheral [48] arteries, and from the evidence that aldosterone stimulates the expression of the NADPH oxidase in macrophages [24]. Cooperation between aldosterone and Ang II has been identified in the release of free radicals that can lead to a deterioration of arterial smooth muscle cells [27]. Although it is generally accepted that the deleterious effects of high concentrations of aldosterone seem to adversely impact blood vessels, the earliest stages of these phenomena are still poorly understood. Weber and his colleagues described an early reduction in intracellular magnesium and calcium concentrations in mono-

cytes and lymphocytes of rats treated with an aldosterone-salt complex [14]. Several markers of oxidative stress were increased in the plasma (alpha-1-antiproteinase activity) and in the heart tissue (gp91phox subunit of NADPH oxidase and 3-nitrotyrosine) of these animals. It may be hypothesized that the early changes in intracellular magnesium and calcium concentrations may also exist in cardiac cells and induce the release of free radicals, coronary lesions, and perivascular (followed by interstitial) fibrosis. It is generally accepted that the generation of oxidative stress is a key event in the progression towards heart failure [4, 10]. Finally, in transgenic mice that overexpress aldosterone synthase in the heart, certain effects have been observed in respect of coronary vasomotricity. In a model characterized by a moderate increase (1.7×) of intra-cardiac aldosterone in the context of unchanged plasma concentrations, the vasodilatory response to acetylcholine is eliminated in male transgenic mice [13]. Another study showed that the damage mechanism involves the inhibition of the BKCa potassium channels of coronary smooth muscle cells [2]. The prevention of coronary alterations by means of eplerenone stimulation thus demonstrates that aldosterone may well bind the MR of coronary cells *in vivo*. Interestingly, both cardiac structure and function remain normal, and the only potentially harmful event discovered to date is the aldosterone-induced coronary alteration itself. In fact, even if coronary responses to acetylcholine may differ between mice and humans [18], these results suggest that a slightly increased aldosterone concentration (reaching a level observed in pathological conditions) can induce coronary dysfunction. This condition may be asymptomatic while the animal is at rest but it may become lethal following an instantaneous increase in cardiac loading. Unpublished data seems to confirm this hypothesis. However, a link to human pathological conditions remains to be established (Fig. 1).

The RALES study provided evidenced that MR inhibition may be beneficial in patients with heart failure. Further is necessary to establish whether the vascular status of these patients can improve over time. This may well prove to be true, since it has been shown that spironolactone improves the endothelial function of patients with heart disease after only one month of treatment [11]. Recent data shows that eplerenone can markedly increase eNOS phosphorylation and thereby improve NO bioavailability in the vasculature. The data suggest that this effect is sup-

Fig. 1. The effects of aldosterone on coronary muscle cells. In an AS transgenic mouse model, aldosterone is produced in cardiomyocytes [13]. Aldosterone binds the MR in coronary smooth muscle cells, decreasing the expression of the alpha and beta subunits of BKCa. This in turn results in a decreased K⁺ efflux, reduced muscle relaxation and a decrease of coronary reserve [2]. This permanent coronary dysfunction may be harmful under conditions of increased coronary flow demand. All of these effects are prevented by eplerenone. Glucocorticoids cannot bind the MR because they are inactivated by the 11-HSD2 enzyme. Blue arrows and blue biochemical compounds indicate the pathways that stimulate BKCa activity. *In vitro* use of Iberiotoxin (a specific inhibitor of the BKCa channel) has helped scientists to better understand this mechanism

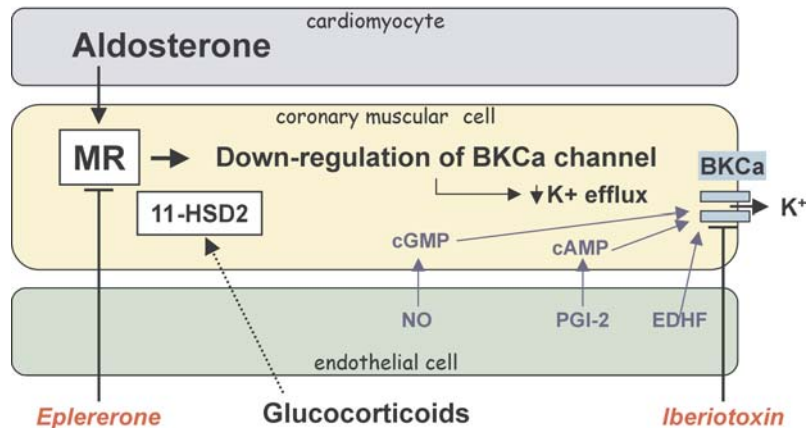
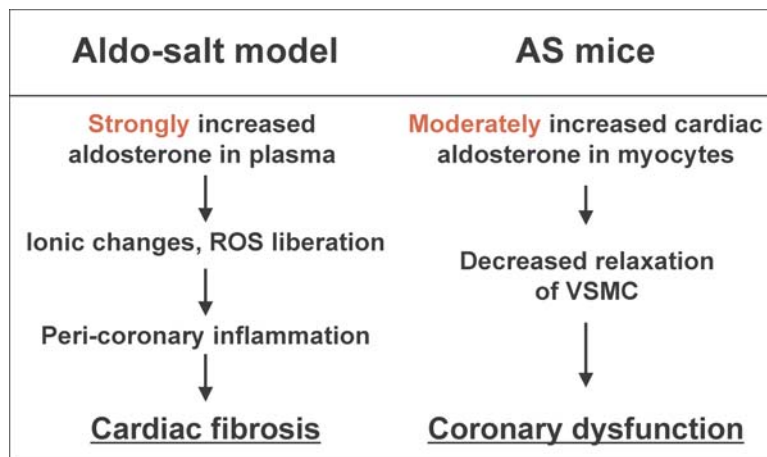


Fig. 2. The effects of aldosterone in the heart are concentration-dependent. The widely-used model of hyperaldosteronism (minipump of aldosterone + NaCl in drinking water [39]) involves a marked increase of plasma aldosterone concentrations. Besides hypertension, the consequences include an inflammatory phenotype which appears rapidly and results in both perivascular and interstitial fibrosis. In contrast, a moderate increase of aldosterone within the cardiac tissue as exhibited by AS mice [13] does not induce such damage. However, it does affect the coronary properties of relaxation in an asymptomatic manner that was not observed with the aldosterone-salt model



plemental to the impact of ACE inhibitors in hyperlipidemic rabbits [21]. A key point is that the beneficial effects of aldosterone blockade in the context of a modulated inflammatory response can be seen very soon after myocardial infarction [12]. Interestingly, an eplerenone-associated reduction in overall mortality was significantly more pronounced in patients from the EPHEsus study who had a history of hypertension. We note that hypertension causes a wide range of vascular alterations [36].

Fibrogenic and arrhythmogenic effects of aldosterone

One of the best-documented effects of aldosterone is the induction of cardiac fibrosis, with adverse conse-

quences on the heart's pump function and accompanying arrhythmogenic effects. A correlation between mortality and the level of initial cardiac fibrosis, and a reduction in cardiac fibrosis after spironolactone treatment, has been observed in a subgroup of patients from the RALES study [49]. Ang II is probably also involved in the onset of fibrosis, since aldosterone increases the density of the cardiac AT1 receptor of Ang II (AT1-R) [39], and the expression of ACE in rat cardiomyocytes [19]. A pro-arrhythmogenic effect of aldosterone (which might partly depend on fibrosis) has been suggested by several studies. In hypertensive patients who exhibit the same degree of hypertension, atrial fibrillation is much more frequent among those who also present primary aldosteronism [30]. In rats with heart disease, spironolactone significantly reduces fibrosis and atrial excitability [29]. Transgenic mice that overexpress MR in cardiomyocytes exhibit normal heart function, but show arrhythmia and sud-

den death [35]. Other effects may be present that can also affect cardiac function. Vassort et al. observed an increase in the slow I_{CaL} calcium current, and a decreased I_{to} transitory potassium current in isolated cardiomyocytes. These phenomena may lead to changes in the electrical characteristics of these cells [5] (Fig. 2).

The signaling pathways of aldosterone

Like all steroid hormones, aldosterone binds to its cytoplasmic receptor, the MR. The hormone-receptor complex dimerizes, migrates into the nucleus and binds to a specific DNA sequence, which in turn triggers the transcription of target genes. In epithelial cells (kidney, colon, salivary glands, skin, etc.), the induced genes include those that encode for the amiloride-sensitive sodium channel ENaC, Na,K-ATPase, and SGK kinase. All of the aforementioned proteins are key factors in the control of sodium reabsorption. The aldosterone-MR complex binds to the glucocorticoid responsive element (GRE). The existence of tissue-specific proteins that modulate the response of the GRE according to the bound hormone (aldosterone or cortisol) has been postulated but not confirmed to date. In vascular cells, a number of target genes have been identified. These include the endothelial NO synthase (NOS3), which is down-regulated by aldosterone (review in [7]), and the smooth muscle cell BKCa, a repolarizing potassium channel whose coronary expression is reduced by aldosterone. The latter is associated with a decrease in the cellular response to acetylcholine and it ultimately leads to a decreased coronary reserve [2].

Besides this mode of action in the context of the classical MR, aldosterone also rapidly induces short-term cellular responses that modulate the concentrations of intracellular Ca²⁺ and cAMP, the activity of the Na-H exchanger, and the phosphorylation of molecules such as the PKC, the EGF receptor (EGFR), and several MAP kinases. These responses are independent of MR activation, as suggested by the rapidity of onset, and several research groups have worked to identify a membrane receptor, without success. There also exists a third mode of action of aldosterone, namely one that is activated by the binding of al-

dosterone to the MR and the subsequent triggering and activation of the EGFR signaling pathway together with an increased phosphorylation of ERK1/2 and JNK 4 kinases [17]. Note that the trigger event occurs in the space of a few minutes (i.e. without protein synthesis). All these mechanisms are associated with the inflammation and vascular remodeling that ultimately leads to fibrosis (Tab. 1).

Tab. 1. The modes of action of aldosterone

	Genomic	Non genotropic	Non genotropic
Time to effect	Hours	Minutes	Minutes
Receptor involved	MR	MR	Membrane receptor?
Aldosterone antagonists	Inhibition	Inhibition	No inhibition
Pathways	Gene transcription	Erk 1/2, JNK	Ca ²⁺ , cAMP

Beyond the well-described genomic mode of action of aldosterone, rapid effects have been observed that are not inhibited by spironolactone or eplerenone (2nd column). Recently discovered [17], a 3rd group of rapid effects involves the MR and is transduced by kinases

The receptors of cortico-steroid hormones

The MR and GR belong to the superfamily of nuclear hormone receptors, and they exhibit a very high sequence homology. The two receptors bind glucocorticoids (cortisol in humans and corticosterone in rats and mice) with strong affinity. Aldosterone binds to MR with a strong affinity, but its affinity for GR is much lower. Because plasma aldosterone levels are three orders of magnitude lower than those of cortisol and corticosterone, glucocorticoids should occupy most of the MRs. However, this theoretical excess is decreased by a factor of 10 due to the binding of glucocorticoids to plasma transcortin (only 3% of cortisol is unbound in the plasma). The binding of aldosterone to albumin is lower (30% of plasma aldosterone is unbound). In addition, transfection studies have shown that cortisol exhibits a transactivation potential of MR that is 10 times lower than that of aldosterone, despite identical binding affinities. In addition, the cortisol-MR complex appears to be less sta-

ble than the aldosterone-MR complex. This may be due to differences in the MR conformational changes that are associated with hormone binding events. This leads to a two to four times faster dissociation of the cortisol-MR complex than that of the aldosterone-MR complex. Finally, the exact mechanisms of entry of cortisol and aldosterone into the cell remain unclear. There may exist other differences between these steroids on account of the aldosterone 11–18 hemiacetal group. One possible conclusion is that aldosterone is at a disadvantage compared to cortisol in respect of MR binding. However, this discrepancy is probably not as pronounced as the ratio of plasma concentrations would suggest. However, under these conditions, it would be interesting to better understand how aldosterone can induce such specific effects.

In epithelial cells and in endothelial and smooth muscle cells, which express the MR, the binding of aldosterone to the MR is made possible by the presence of the enzyme 11-HSD2 (11-beta-hydroxysteroid dehydrogenase type II), which metabolizes cortisol and corticosterone into their inactive cortisone and 11-dehydro-corticosterone metabolites. In contrast, in cells such as cardiomyocytes, which express the MR but not the 11-HSD2, the MR is probably mostly occupied by glucocorticoids. Given this hypothesis, aldosterone probably exhibits no significant MR-dependent activity. However, as outlined above, there are several possible alternative mechanisms that may permit the binding of aldosterone with the MR, even in absence of 11-HSD2. Studies that have explored isolated cardiomyocytes provide evidence for the effects of aldosterone on calcium or potassium currents. However, these effects have only been observed using high concentrations of aldosterone in a milieu that contains a minimal concentration of glucocorticoids. Notwithstanding the possible existence of an as yet unknown mechanism of aldosterone selectivity, it appears that effects may either exist in an attenuated form *in vivo*, or they are a consequence of MR activation in a manner that is independent of the activating hormone.

The situation is different for the cortisol-mineralocorticoid receptor complex. Under physiological conditions, this complex is inactive but, under pathophysiological conditions, it may be activated whereupon it begins to function like the aldosterone-mineralocorticoid receptor complex [47]. It would be interesting to compare the effects of aldosterone and those of glucocorticoids in the presence of a GR inhibitor in

order to “see” only the MR-dependent effects on isolated cardiomyocytes. One might hypothesize that they would be identical, since both hormones can bind with MR with the same affinity. In this sense, a recent article shows that corticosterone activates the MR in smooth muscle cells and triggers rapid responses in terms of the MAP kinase and ERK1/2 pathways, both of which may have adverse consequences on the vessel [33]. In addition, several studies have shown that cortisol may block the action of aldosterone, which in turn suggests that in many cases, cortisol binds the MR and acts as a MR antagonist. Transgenic mice that overexpress the MR in cardiomyocytes [35] or in other cells are a powerful and elegant means to explore these mechanisms. We emphasize that MR antagonists can inhibit the actions of aldosterone in cells that express 11-HSD2, but they may also inhibit the action of cortisol in cells that do not express this enzyme. In the case of the poorly specific MR antagonist spironolactone, the GR-mediated activity may also be partially inhibited.

Interference between aldosterone and angiotensin II

One of the difficulties in interpreting the effects of aldosterone depends on the interactions between the signaling pathways activated by other hormones or receptors, namely Ang II and MR (review in [26]). Several laboratories have demonstrated that aldosterone stimulates the transcription of AT1-R, and of the Ang II converting enzyme ACE, which results in an increased local production of Ang II. On the other hand, the Ang II-dependent increase in collagens is at least in part an aldosterone-dependent effect [34]. It has been recently shown in hamster isolated cardiomyocytes that eplerenone inhibits the intracrine action of Ang II on inward calcium currents and drastically reduces the effects of extracellular Ang II on the ICa current [8]. Since eplerenone reverses the effects of aldosterone, these results seem to suggest that the MR is an essential component of the intracrine renin-angiotensin-aldosterone system. Interestingly, the proliferation of vascular smooth muscle cells is stimulated by a combination of very low doses of aldosterone and of Ang II, while aldosterone or Ang II alone have no effect at these doses [31]. Similarly, aldoster-

one increases neovascularization in an *in vivo* model of ischemia secondary to right femoral artery ligation in mice [28]. Inhibition of these effects by valsartan suggests that the pro-angiogenic action of aldosterone may involve the AT1-R. Finally, Ang II can directly activate the MR in both coronary and aortic SMC [22]. Accordingly, there are interactions between the effects of aldosterone and those of Ang II. This emphasizes the therapeutic potential of combining MR and AT1R inhibitors to treat cardiovascular pathologies.

Conclusion

Our understanding of aldosterone is far from complete. In addition to the mechanisms, it is important to determine whether the increase in aldosterone in common diseases such as diabetes, hypertension and left ventricular hypertrophy is an additional risk factor for patients. This question also arises in studies of the metabolic syndrome, another situation in which plasma aldosterone is increased [25]. In rat cardiomyocytes, locally produced aldosterone modulates potassium currents and increases oxidative stress, but only in male diabetic animals [41]. However, preliminary results from our laboratory show that in the mouse heart, a slight increase in aldosterone concentrations can exert a protective influence against the deleterious effects of type 1 diabetes. Another example of the complexity of the effects of aldosterone is the reality that the outcomes seem to vary depending of the concentration of the hormone, the gender of the specimen/subject, and the nature of the local cellular environment.

Acknowledgments:

This work was supported by Inserm, CNRS, and the Fondation de France.

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Received:

October 6, 2008; in revised form: January 27, 2009.