A hypothesis about the potential role of statin administration as adjuvant treatment in the management of Merlin-deficient tumors

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Keywords:
Merlin-deficient tumors
Rho-GTP-ases
Statins

A R T I C L E   I N F O

Article history:
Received 5 December 2013
Revised 4 February 2014
Accepted 17 February 2014

A B S T R A C T

Merlin, a tumor suppressor protein, controls essential steps of cell cycle, and its deficiency results in cellular overgrowth, proliferation, angiogenesis, invasion and metastasis. Lack of Merlin is responsible for neurofibromatosis-2, most schwannomas, and many meningiomas and ependymomas. We hypothesize that there is a role for statins to ameliorate Merlin’s deficiency in this set of tumors by inhibiting a number of Merlin’s downstream effectors, the small Rho-GTP-ases, and we present the relevant data. The ultimate goal is to offer a medical therapy promising to halt or reduce the tumor growth-rate in patients harboring Merlin-deficient neoplasms and to provide an adjuvant systemic therapy for patients undergoing stereotactic radio-surgery and partial tumor resection.

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Introduction

Meningiomas and schwannomas are benign central nervous system tumors, and constitute 40% and 10% of all primary intracranial tumors, respectively [1]. Moreover, ependymomas, which are malignant primary CNS tumors, represent 10% of the posterior fossa tumors in children [2]. All these types of CNS tumors have been associated with neurofibromatosis type-2 (NF-2), a familial multiple neoplasia syndrome. It is inherited in an autosomal dominant manner, and demonstrates 100% penetrance by the 60th year of age [3,4]. Its incidence has been reported to be as high as 1/25000 live births [5]. Its cranial manifestations include bilateral vestibular schwannomas, and multiple and aggressive meningiomas and ependymomas [5,6]. It has been demonstrated that Merlin-deficient tumors are frequent and can be multiple in certain patients [1,7]. The tumor suppressor Merlin is a regulatory mediator of the cell cycle [8–10]. Its deficiency has initially been associated with neurofibromatosis-2 (NF-2) [9–11]. Today, germ-line mutations of the NF-2 gene have been described in 45–66% of sporadic schwannomas [12,13], 60% of sporadic meningiomas, and 33% of sporadic ependymomas [13]. Embryos with homozygous mutations of the NF-2 gene die in utero [14].

Multiple treatment modalities have been employed in the management of the above mentioned neoplastic entities, either in their NF-2 associated or in their sporadic forms. These include extensive, surgical resection, conventional radiotherapy and stereotactic radio-surgery [6,15,16]. All these disciplines carry inherent high morbidity and considerable mortality risk [6]. New onset damage to the facial and vestibulocochlear nerve are among the most common postoperative complications in vestibular schwannoma surgery, with a reported frequency to be as high as 38% and 87%, respectively [17]. Moreover, the local nature of surgery and stereotactic radio-surgery limits their usefulness in patients with multimodal NF-2 [1]. Therefore, the development of new treatment strategies is critical for managing these patients, and improving their survival and their quality of life.

There is a growing body of evidence in the literature that the absence of Merlin can be ameliorated by the administration of statins, a group of drugs commonly employed in the prevention and treatment of primary and secondary ischemic heart disease. However, there is no literature-evidence, to the best of our
knowledge, supporting the use of statins in Merlin-deficient tumors. In our current communication, we hypothesize that statins can be used in the prevention and management of these tumors, and we provide the relevant evidence supporting this theory.

**Merlin and tumor suppression**

The NF-2 gene, identified in 1993, is a tumor suppressor gene located on chromosome 22q12 and encodes a 69 kDa protein [8–11]. Its gene product, known as “Merlin” (Moesin-Erzin-Radixin-like protein) or “schwannomin” [15,16,18], belongs to a greater protein super-family, the Band 4.1 protein family, which includes band-4.1-erythrocyte protein, ezrin, radixin, and moezin proteins. These proteins usually link the actin cytoskeleton to the cell surface glycoproteins, to control cell growth, remodelling, motility and invasion [15,16,18]. It exists in ten isoforms, with isoform-1 and −2 being the most common. All isoforms possess an N-terminal part, which contains the four point one Erzin-Radixin-Moezin (FERM) domain, followed by an alpha-helical domain, and a hydrophilic tail [18].

Merlin is involved in the control of various aspects of the cell cycle and the tumorigenesis (Fig. 1) [4,19,20]. It suppresses aberrant cell-growth and proliferation by promoting the endocytosis of growth-factor membrane receptors [19,21] and by controlling the transcription/translation of Cyclin-D, a key cell-regulator of G1 phase and cell cycle [19,21–23]. It also demonstrates a pro-apoptotic effect of the senescent cell by inhibiting the Ra-1 guanine nucleotide dissociation stimulators (Ra-1 DGs) [20]. Moreover, Merlin has a protective role against tumor invasion and metastasis through contact inhibition at the tight and adherent junctions, and also by regulating actin cytoskeletal dynamics responsible for the formation of lamellipodia [4,24–29]. Furthermore, it has been demonstrated that Merlin regulates angiogenesis in normal cells by means of semaphorin 3 F [30].

A common feature among all these pathways is the pivotal role of proteins belonging to the Ras superfamily [31]. These are small GTP binding proteins, which consist of five subfamilies: Ras, Rho, Rab, Ran and Arf [31]. The Ras family mediates extracellular signals into the cell, and controls gene transcription, cell growth and differentiation. In humans there are Ras oncogenes that regulate cell proliferation, differentiation morphology, and apoptosis [31]. The Rho subfamily includes, among others, the RHO, RAC, and CDC42 proteins, and is involved in cytoskeletal organization, cell polarity, gene expression, and cell cycle progression [31]. The Rab and Raf families are involved in endocytic and secretory functions, while the Ran family controls nuclear transport [31]. All members have been evolutionary preserved across several phyla, and possess an N-terminal with GTP-ase properties and a C-terminal [31].

The N-terminal switches from an active (GTP-bound) form to an inactive (GDP-bound) [31]. The balance between these two forms is mediated by two groups of regulatory molecules. The first group,
the guanine-nucleotide exchange factors (GEFs), activated by various signals, promote the formation of the active form. The second group, the GTP-ase activating proteins (GAPs) promote the hydrolysis of the active to the inactive form [32]. Moreover, these proteins require either geranylgeranylation or farnesylation or both at their C-terminal before reaching their mature form, a process which takes place during their post-translational modification. This process is essential for proper intracellular localization and function [33]. However geranylgeranylation and farnesylation are part of the HMG-CoA pathway, whose limiting step is enhanced by HMG-CoA and is blocked by statins [34].

The interplay between the Rho-GTP-ases and Merlin becomes even more interesting as Rac and Cdc-42 control the activation status of Merlin (Fig. 2). The latter oscillates between an active and an inactive form, by the phosphorylation on serine-518, a reaction mediated by p21 activated kinase (PAK) [24]. However, PAK is a downstream product in the Rac and Cdc42 pathway [4].

Knock-down of both NF-2 gene alleles is required in Merlin-deficiency states according to the two-hit-hypothesis of Knudson [35]. A pathologic gene allele might occur either de novo or be inherited. Mutation of the second allele will result in a pathologic cell-lineage, responsible for the patient’s phenotype in susceptible organ systems [15,16].

Our hypothesis

Our hypothesis is to use the statins in order to ameliorate the absence of Merlin in Merlin-deficient tumors (Fig. 3). Merlin controls the cell cycle by inhibiting the small Rho-GTP-ases. In the absence of Merlin, the latter act in an uncontrolled manner leading to tumor development. Statins blockade of the mevalonate pathway would inhibit the biosynthesis of small Rho-GTP-ases, Rho, Ras, Rac, and Cdc-42, parallel to the inhibition of cholesterol. Inhibition of the small Rho-GTP-ases biosynthesis would downregulate both the external and internal growth signals, permit contact inhibition, slow cellular proliferation, control angiogenesis, invasion, and metastasis. In case of any remaining Merlin effect, the statin administration would enhance its action by unmasking its suppression by the small Rho-GTP-ases. In other words, statins would take the lost control of the cell cycle instead of Merlin.

The aim of administering statins in patients with Merlin-deficient tumors is to halt or slow the progression of these neoplasms, decreasing thus, the total number of surgery or radiotherapy candidates. Statins can also be used as an adjuvant therapy in cases with partial tumor resection or stereotactic radiosurgery. Furthermore, NF-2 patients would receive a systemic therapy, in addition to the regional treatments, that potentially would improve their quality of life and increase their life expectancy.

Statins

Statins are a group of substances which inhibit the enzyme HMG-CoA reductase, and as a consequence are potent suppressors of cholesterol biosynthesis [36]. They were developed in the 1980’s after noting that a fungal metabolite (mevastatin, compactin) could
inhibit a critical step in the melavonate pathway lowering thus, the circulating levels of endogenous cholesterol [37,38]. Up-to-date, nine statins have been studied extensively, eight of which are currently used (lovastatin, mevastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, rosuvastatin and pitavastatin) in clinical practice, and one was withdrawn (cerivastatin) due to reported side effects [39]. Their pharmacokinetic and pharmacodynamic properties and their safety profile are important parameters in their consideration in the management of Merlin-deficient tumors.

Statins are orally administered, with considerable variations regarding their dosage (10-80 mg/day), hydrophilic profile, tissue availability, and metabolism. Among them, pravastatin and rosuvastatin are extremely hydrophilic, atorvastatin, simvastatin, pitavastatin and lovastatin are hydrophobic, while fluvastatin is somewhere in the middle [40,41]. All statins exhibit significant liver first-pass effect. The cytochrome 450 plays a pivotal role in most hydrophobic statin metabolism, in contrast to the hydrophilic statins and pitavastatin [41-44]. Statins (with the exception of pravastatin) are largely bound to serum proteins, and thus the resulting systemic exposure to the unbound active substance is minimal [41]. Simvastatin and lovastatin are the most potent drugs in terms of crossing the blood brain barrier, achieving effective concentrations in the cerebrospinal fluid, with fluvastatin and pravastatin being on the other edge [45-48].

Nowadays, statins are widely used in the primary and secondary treatment of coronary artery disease, diabetes mellitus, ischemic stroke, and renal dysfunction [49,50]. Their primary target-organ is the liver, which mediates their action mainly by lowering the total and LDL cholesterol. Their lipid lowering properties are manifested in nanomolar serum concentrations and are achieved by their structural similarity to HMG-CoA and their reversible competitive binding to the HMG-CoA reductase [36,38]. In addition, there is abundance of evidence indicating that statins’ beneficial function is largely cholesterol-independent [49]. These collateral benefits are known as the “pleiotropic effects of statins” and are manifested by inhibition of the biosynthesis of isoprenoids [34]. The latter affect the post-translational modifications of intercellular signaling molecules and can be classified as anti-inflammatory, immunomodulatory or anti-neoplastic [36,49,51,52]. The pleiotropic effects have been reported to occur at higher concentrations [52], and provoke the expansion of the listed indications on the use of statins in a number of other conditions including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, subarachnoid hemorrhage, traumatic brain injury, medulloblastomas, and pancreatic cancer.

The safety profile of statins is excellent, with myalgia being the commonest reported adverse effect (7%), followed by myopathy (<0.2%), rhabdomyolysis (<0.05%), diabetes mellitus, and asymptomatic transaminasaemia to a lesser extent [44,53]. Despite the fact that most of these side effects are dose-dependent, statins are well tolerated in higher doses [44]. In addition, statins present important interactions with other drugs that share the same enzyme substrate, such as antiretrovirals, calcium channel blockers, and genefibrole [44,53].

**From bench to bedside**

Recent in vitro studies support our hypothesis. Lovastatin inhibits the proliferation of meningioma cells in vitro in a dose dependent manner, an effect that is partially reversed by the administration of melavonate [54]. Meningioma cell-lines have been reported to be sensitive to the cytotoxic effect of simvastatin, which is moderately enhanced by the addition of pioglitazone [55].

However, before moving from theory to clinical praxis and administer statins to patients with Merlin-deficient tumors, a few important parameters should be controlled. Are Merlin-deficient schwannomas and ependymomas inhibited by statins in vitro? Are statins effective in Merlin deficient tumors in vivo, as well? If yes, which statin and in what dose should be preferred? It is reminded that the pleiotropic effects are substance- and dose-dependent [55]. The chosen statin should cross the blood brain barrier and maintain sufficient concentration in brain tissue and cerebrospinal fluid, with the least systemic toxicity, in order to achieve the desired results. It is expected that the anti-neoplastic effect would be achieved with high-dose statin regimen [56]. In addition, controlled studies comparing the tumor progression in patients on statins with controls (or the disease > natural history) would justify our hypothesis. Finally, another set of studies would select the optimal target population in terms of age, disease stage, and de-novo versus inherited cases.

A number of difficulties are well anticipated in testing our hypothesis. Many of the biochemical pathways that describe Merlin’s function are at an investigational level. Similarly, there is still confusion regarding the pleiotropic effects of statins. On the other hand, highly specialized laboratories are needed to culture Merlin-deficient schwannoma and meningioma cell lines and test the cytotoxicity of statins. In addition, the in-vivo testing of our hypothesis demands the participation of a large patient sample for a long time, undergoing expensive investigations (magnetic resonance imaging), repeatedly. Things are more difficult in the case of NF-2 patients due to their rarity and lesion multiplicity. Only special referral centers come across a sufficient number of NF-2 patients for a sufficient time in order to perform the above noted studies. Finally, surgery is unavoidable for the present, since the surgical access to the tumor cells is mandatory in order to verify Merlin’s deficiency, downgrading statin’s role to adjuvant therapy.

Concluding, there is a potential role of statins in the management of Merlin-deficient tumors based on their pleiotropic effects. However, it is expected to be manifested by statins that cross the blood brain barrier and in higher doses. The aim is to halt or slow these tumors, especially in NF-2 patients. However, further studies are needed to validate the present hypothesis.

**References**
