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Mini Review

The role of AGR2 and AGR3 in cancer: Similar but not identical

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ABSTRACT

In the past decades, highly related members of the protein disulphide isomerase family, anterior gradient protein AGR2 and AGR3, attracted researchers' attention due to their putative involvement in developmental processes and carcinogenesis. While AGR2 has been widely demonstrated as a metastasis-related protein whose elevated expression predicts worse patient outcome, little is known about AGR3's role in tumour biology. Thus, we aim to confront the issue of AGR3 function in physiology and pathology in the following review by comparing this protein with the better-described homologue AGR2. Relying on available data and *in silico* analyses, we show that AGR proteins are co-expressed or uncoupled in context-dependent manners in diverse carcinomas and healthy tissues. Further, we discuss plausible roles of both proteins in tumour-associated processes such as differentiation, proliferation, migration, invasion and metastasis. This work brings new hints and stimulates further thoughts on hitherto unresolved conundrum of anterior gradient protein function.

Introduction

Anterior gradient (AGR) proteins form an evolutionarily broad family with prominent, however, poorly understood physiological functions in vertebrates. The first identified was a secreted protein XAG-2, originally discovered in *Xenopus laevis* and implicated in the formation of the anteroposterior axis during embryogenesis (Sive et al., 1989). AGR2 extracellular role was also documented during limb regeneration in salamanders by stimulation of blastemal growth (Kumar et al., 2007). In addition, it was recently demonstrated that AGR proteins in *X. laevis* promote regeneration of hindlimb buds and tails of tadpoles (Ivanova et al., 2013). During the last decades, intense research has commenced in order to elucidate biological function of human homologues, namely AGR2 and AGR3 both in health and disease. Strikingly, although both molecules share 71% sequence identity and lie adjacent to one another at chromosomal position 7p21 (Fletcher et al., 2003; Petek et al., 2000), AGR2, but not AGR3, is a dominant factor identified in many OMICS screens, and therefore, many more reports have been published in relation to its characterization. Thus, relying also on our recent observation of uncoupled AGR2 and AGR3 expression in various tumour tissues (unpublished data), we sought to compare both proteins by analysing their expression pattern and regulatory mechanisms in this review. For this reason, we have reviewed all the published data and used the Genevestigator tool (Hruz et al., 2008), which enables not only prediction of AGRs tissue distributions but also their co-expressed partners. Moreover, we performed *in silico* promoter analysis to find upstream regulatory pathways potentially triggering AGR2 and AGR3 expression. All the analyses were conducted on the data available online in May 2014.

Abbreviations: AA, amino acids; ALS, familial amyotrophic lateral sclerosis; AGR, anterior gradient; AhR, aryl hydrocarbon receptor; AR, androgen receptor; CAPN8, calpain 8; CAPN9, calpain 9; CDDP, cisplatin; CFTR, cystic fibrosis transmembrane conductance regulator; CLDN3, claudin 3; DAG-1, alpha-dystroglycan; EGFR, epidermal growth factor receptor; ELK1, ETS-domain containing protein; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; EsR, oestrogen receptor; FOX, forkhead box; GC-SBE, glycine-rich SMAD binding elements; HCC, hepatocellular carcinomas; HGSC, high-grade serous ovarian carcinomas; ICC, intrahepatic cholangiocarcinomas; JAK-STAT, Janus kinase-signal transducer and activator of transcription; LEF-1, lymphoid enhancer factor-1; LGSC, low-grade serous ovarian carcinomas; MAPK, mitogen-activated protein kinases; MICA, MHC I-related chain A; MUCs, mucins; NK, natural killer; PDB, Protein Data Bank; PDIs, protein disulphide isomerases; SLC44A4, protein member 4 of solute carrier family 44; TGF-β, transforming growth factor-beta; TGIF1, TG-interacting factor 1; TM4SF, transmembrane 4 superfamily; TMC4, transmembrane channel-like protein 4; TMC5, transmembrane channel-like protein 5; TMPRSS2, transmembrane protease serine 2; TRX, thioredoxin; TSPAN1, tetraspanin 1; TSS, transcription start site; UPR, unfolded protein response; ZEB1, zinc-finger enhancer binding-1.

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AGR2 and AGR3 as PDI family members

Structural features of the PDI family

The protein disulphide isomerase (PDI) family is part of the thioredoxin (TRX) superfamily, which also includes the glutaredoxins, thioredoxins, ferroredoxins and peroxiredoxins (Jacquot et al., 2002). Recent work has revealed that there are three subfamilies of AGRs: AG1, AGR2 and AGR3, all showing the highest homology to non-secreted PDI of the TLP19 subfamily. Remarkably, members of AGR2 and AGR3 subfamilies are present in amniotes, while members of AG1 subfamily (to which family founder, XAG-2 belongs) are restricted to lower vertebrates (Ivanova et al., 2013). Human AGR2 and AGR3's affiliation to the PDI family was based on the phylogenetic analysis performed by Persson et al. (2005), where it was shown that both proteins have high homology to ERp18/19 protein, also denoted as AGR1. To date, 21 members varying in size, structure, tissue distribution and enzymatic activity were identified (Galligan and Petersen, 2012). The common feature of all the PDI members is the presence of at least one domain with structural similarity to TRX, which can be either active or enzymatically inactive (Kozlov et al., 2010). Protein activity depends on the presence of CXXC motif, which determines the reaction with thiols of newly synthesized proteins. However, some of the proteins vary in their active site composition, including AGR2 and AGR3 proteins with CXXS motif (Galligan and Petersen, 2012); therefore, it is possible that in order to act as PDIs AGR proteins cooperate with other redox-active molecules. Within the family, ERp18, TMX, TMX2, TMX4, TMX5, AGR2 and AGR3 proteins possess a single active domain, while ERp27, ERp29, CASQ1 and CASQ2 proteins contain only inactive domains (Benham, 2012; Galligan and Petersen, 2012). Moreover, each protein of the family is characterized by the presence of a short NH_2-terminal signal peptide and the COOH-terminal endoplasmic reticulum (ER) retention sequence (with the exceptions of TMX1, TMX4, CASQ1 and CASQ2 that lack the ER retention sequence) (Appenzeller-Herzog and Ellgaard, 2008; Galligan and Petersen, 2012). An ample insight into the sequence characterization as well as structural overview of the PDI family can be found in the following works (Appenzeller-Herzog and Ellgaard, 2008; Galligan and Petersen, 2012; Kozlov et al., 2010).

The structure of the mature AGR2 protein, characterized by the lack of the first 20-amino acid signal peptide, was recently characterized. It was shown that unfolded N-terminal 21–40 amino acid region determines adhesion properties of AGR2, whereas the folded domain forms a dimer through specific intermolecular salt bridges. Moreover, in that work, the authors demonstrated that the proper topography of the dimeric structure relies on interaction between amino acids E60 and K64 (Patel et al., 2013). Relying on available dimeric structures of AGR proteins in Protein Data Bank [PDB accession numbers—AGR2: 2LNS, AGR3: 3PH9], it can be concluded that AGR2 secondary structure consists of α-β-α-α-β-α structural motifs, whereas AGR3 consists of α-β-α-α-β-α-β-α-α-α motifs. AGR2 and AGR3 thioredoxin domain with active site CPHS and CQYS, respectively, is situated on α2 helix. The main difference between AGR2 and AGR3 structure lies in the dimerization region (Fig. 1). The dimerization of AGR2 arises from interaction between random coils (residues 45–54) and α1 helices (between residues 60–67), whereas AGR3 is predicted to form dimer through random coils corresponding to 32–36, 42–46 and 100–105 residues as well as part of α1 helix (residues 47–53). Moreover, based on PDBsum database, we suggest that AGR3 dimerization occurs through specific hydrogen bonds between amino acids Q32 and Q46. However, to verify whether AGR homologues are able to dimerize with each other through random coils and/or α1 helices warrants further investigation.

Role of PDI family in cell homeostasis

The PDI family members are implicated in a variety of disorders, including neurodegenerative syndromes such as Parkinson's, Alzheimer's, Huntington's diseases, familial amyotrophic lateral sclerosis (ALS) as well as infertility and a diverse range of malignancies (as reviewed by Benham, 2012). Of note is also a report showing decrease of AGR2 expression in ulcerative colitis, where it was postulated to play a crucial role in the maintenance of epithelial integrity (Zheng et al., 2006).

The main function of the PDI family members is to form/disrupt, oxidize/reduce and isomerize the disulphide bonds between the cysteine residues of nascent proteins in the lumen of the endoplasmic reticulum (ER) in order to provide their proper folding and maturation prior to the release for cellular transport (Hatahet and Ruddock, 2009). Thus, they play a pivotal role in the maintenance of cellular homeostasis. They can also serve as molecular chaperones involved in the ER-associated degradation (ERAD) mechanisms that lead to protein removal (Ni and Lee, 2007). Apart from their ubiquitous expression in ER lumen, PDIs can be found in other cellular compartments, where they are shown to regulate among others cell adhesion, platelets activation, viral infections and protein–DNA interactions (Turano et al., 2002).

Although oxidative properties of AGR2 protein have not yet been validated *in vitro* or *in vivo*, some AGR2 client proteins have been identified, supporting its role in the protein folding machinery. For instance, AGR2 was demonstrated to regulate production of mucins (MUCs), including intestinal MUC2 (Park et al., 2009), the airway epithelial MUC5AC and MUC5B (Schroeder et al., 2012) as well as pancreatic MUC1 (Norris et al., 2013). Additionally, by forming a substrate loop between amino acids 104 and 111, it interacts with ATP-binding protein Reptin and consequently regulates many of its functions such as ATPase activity, ATP binding, helicase functions, telomerase/Pontin binding and others (Maslon et al., 2010). Further, AGR2-interacting proteins were identified in yeast two-hybrid screen, including neurexin 3, cytoskeleton-associated protein 2 or Ly6/PLAUR domain-containing protein 3, linking AGR2 with cell adhesion, division and migration (see review by Chevet et al., 2013).

Perturbation of ER homeostasis leads to the accumulation of unfolded or mis-folded proteins, ER stress and consequently activation of the unfolded protein response (UPR). UPR signalling results either in the degradation of mis-folded proteins by upregulating PDIs and molecular chaperones or in the attenuation of protein synthesis (Ron and Walter, 2007). However, if ER homeostasis cannot be restored, apoptotic pathways are induced (Tabas and Ron, 2011). Using both proteomic and biochemical approaches, Higa et al. identified AGR2 as one of the ER proteins that associates with membrane-bound ribosomes through nascent protein chains. They found that AGR2 is induced upon ER stress and that its basal expression is controlled by the IRE1α- and ATF6α-triggered arms of the UPR. They also showed that AGR2 silencing altered the expression of ERAD components, resulting in cell survival under stress condition (Higa et al., 2011). Additionally, an independent study showed that AGR2 homo-dimerization is important for the association with BiP/GRP78, a well-established chaperone involved in the cellular response to many stresses (Ryu et al., 2013).

AGR2 and AGR3 expression in tumour cells and tissues

Uncoupled expression of AGR proteins in carcinomas

Both AGR2 and AGR3 were originally found in breast cancer specimens. *AGR2* gene was first described in the oestrogen receptor (EsR)-positive MCF-7 cell line (Thompson and Weigel, 1998), and AGR3 protein was identified in the membrane of breast cancer

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