Volume 58(1):73-94, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Anaerobic biodegradation of cellulose-rich substrates

Orsolya Strang

Department of Biotechnology, University of Szeged, Szeged, Hungary

Depletion of fossil fuels and increase of global climate changes demand the usage of renewable energy sources. Biogas forms anaerobically during the decomposition of different organic materials. In the process, three syntrophic groups of microbes work together. These are the polymer degraders, acetogens and methanogens. The main components of the produced biogas are methane (55-70%) and carbon-dioxide (30-45%). After upgrading, biogas could be injected into natural gas grid. For biogas production, many waste and raw material is suitable. Plant biomass is the largest amount of biomass on Earth. Plants can harvest solar energy during photosynthesis and convert it to plant tissues, therefore have vast energy potential. Plant tissues consist of lignocellulose as the major component. Lignocellulose is composed of cellulose, hemicellulose and lignin. Cellulose is a recalcitrant complex polymeric carbohydrate, cellulases are needed for its efficient decomposition. Cellulases are divided into three major groups: endoglucanases (EC 3.2.1.4), exoglucanases (3.2.1.91) and β-glucosidases (3.2.1.21). Endoglucanases cut at random internal sites into the cellulose polysaccharide chain, generating new chain ends. Exoglucanases act on the reducing or nonreducing ends of cellulose, liberating glucose or cellobiose units. β-glucosidases hydrolyze cellodextrins and cellobiose to glucose which can be used in metabolic pathways.

For the utilization of substrates having high cellulose content – without pretreatment - the biogas producing microbial community should contain a significant number of cellulose producing bacteria and they should break down cellulose to easily utilizable sugar monomers. An adaptation strategy to adapt the community to lignocellulosic substrate has been developed. The experiments were carried out under thermophilic conditions at 55 °C. α-cellulose was used as substrate for the adaptation and the control fermentors received glucose as carbon and energy source. The changes in the concentration of volatile fatty acids were followed by HPLC, the β-glucosidase enzyme activity was monitored regularly. From the adapted microbial community, cellulose degraders were isolated and were also used as inoculum in the next set of biogas experiments. The cellulose degrading microbes had positive effect, elevated the biogas and methane yield. DNA was purified from the cellulose degrading consortia and was undergone metagenome analysis. In the thermophilic cellulose degrading consortium, the main orders were Thermoanaerobacterales (70%) and Clostridiales (10%). *Thermoanaerobacterium thermosaccharolyticum*, *Caldanaerobacter subterraneus*, *Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum* were identified as dominant strains.

Supervisor: Kornél L. Kovács, Zoltán Bagi
E-mail: strangorsi@citromail.hu

Uniform or different? Heterogeneity of murine bone marrow mesenchymal stem cells in differentiation and immunosuppression

Enikő Szabó

Lymphocyte Signaling Group, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Bone marrow mesenchymal stem (BMMSCs) are adherent, colony-forming cells and are defined as multipotent cells differentiating into several cell types (e.g. osteoblasts, chondrocytes and adipocytes). BMMSCs have been found therapeutically beneficent in models for numerous human diseases by multiple processes including enhancement of tissue regeneration, supporting angiogenesis, subduing inflammation and modulating the immune response at the site of tissue damage. Despite the incessantly increasing number of preclinical and clinical MSC studies, there are some basic issues about MSCs, which still remain unresolved. The heterogeneity in differentiation potential of MSCs was demonstrated decades ago. Up to this day, few and inconsistent data have been collected reporting uniform or different immunosuppressive properties of single MSC clones, even if it is highly relevant to the therapeutical effectivity of MSCs.

We aimed to examine the heterogeneity of murine BMMSC population through characterizing 6 single cell-derived MSC clones (MSC1-MSC6) in terms of differentiation potential, support of angiogenesis and immunmodulation.

To examine whether MSC clones maintain the multipotency of BMMSC population, MSC clones were induced to differentiate *in vitro* into adipocytes and osteoblasts. While MSC2-6 differentiated into both lineages, MSC1 differentiated only into adipocytes.

Analysis of the ability of the MSC clones to support angiogenesis has been carried out using an *in vitro* model, the capillary mimicry assay. MSC clones were co-cultured with H5V endothelial cells and the capillary-like structures were evaluated. Whereas neither MSCs nor H5V formed capillary-like structures alone, all MSC clones supported similarly the development of these structures when co-cultured with H5V.

The *in vitro* immunmodulatory properties of BMMSC clones were compared in ConA-stimulated T cell proliferation assay. MSCs were co-cultured with T cells isolated from mouse lymph nodes in the presence of ConA and cell division of CFSE (a fluorescent dye used for proliferation assays) labeled T cells was followed by flow cytometry. All MSC clones inhibited significantly but not uniformly the T cell proliferation in the following order: MSC2>MSC4=MSC5>MSC1>MSC3>MSC6. Differences in the inhibition of T cell proliferation

were reflected in expression level of *Nos2, Ptgs2*, the most important genes responsible for murine MSC-mediated immunosuppression. The strongest inhibitor MSCs expressed the most and the least inhibitor clones expressed the lowest level of these factors at mRNA level. Normally, MSCs exert immunosuppression at the site of inflammation, therefore the immunmodulation of MSC clones were tested in inflammation-mimicking milieu, treating MSC clones with pro-inflammatory cytokines, IFN-γ and TNF-α. Treatment of the cells with these cytokines resulted in upregulation of *Nos2* and *Ptgs2* gene expression in each MSC clone, and as a consequence, their inhibitory effect on T cell proliferation elevated. To find out whether MSC clones can exert immunmodulation *in vivo,* the effect of the most and the least immunosuppressive MSC2 and MSC6 clones, respectively, were tested in ovalbumin-induced delayed-type hypersensitivity response in mice. Intraperitoneal administration of MSC2 cells simultaneously with ovalbumin immunization significantly reduced, whereas MSC6 didn't change the ovalbumin-induced increase of footpad thickness, unless MSC6 cells were pretreated with IFN-γ and TNF-α prior to injection, in that case MSC6 also decreased footpad thickness increment vigorously.

Based on our results, we suggest that murine BMMSC population is homogenous in differentiation and angiogenesis support while heterogeneous in immunosuppression. Dissimilarity in the immunosuppressive function likely depends on the activation state of single MSC cells, since placing the cells into an inflammatory milieu, the immunomodulatory effect of different MSC clones becomes similar.

Supervisors: Monostori Éva, Czibula Ágnes
E-mail: szabo.eniko@brc.mta.hu

Genetic analysis of *Saccharomyces cerevisiae RAD5* gene

Róbert Tóth

Mutagenesis and Carcinogenesis Research Group, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

The sequence of DNA contains important information for the life of cells. Any damage of DNA leads to inaccurate function of the cell, or occasionally to its death. Therefore, proteins of DNA repair have a critical role in preserving the initial state of DNA.

At DNA damage, the replication polymerase stalls and the complex of Rad6 and Rad18 proteins ubiquitylates the Proliferating Cell Nuclear Antigen (PCNA), the processivity factor of replication polymerases, at its lysine 164 residue. Subsequently, the monoubiquitylated PCNA is polyubiquitylated by the protein complex of Rad5, Mms2 and Ubc13.

The Rad5 has three domains: RING, Helicase/ATPase and Hiran domain. While the RING domain has E3 ubiqutine ligase activity and the Helicase/ATPase domain has a replication fork reversal activity facilitating the formation of a chicken-foot DNA structure, the function of the Hiran domain is unknown despite of its predicted DNA binding capability.

To explore the particular functions of these Rad5 domains we tested the sensitivity of *ring* (CC914,917AA), *atpase* (DE681,682AA) and *ring-atpase* double point mutant strains with different mutagenic agents such as UV-light, methyl methanesulphonate and nitrogen-mustard.

We have found that the single mutant strains (*ring*, *atpase*) were sensitive to all tested mutagens and the double mutant strain (*ring-atpase*) had a higher sensitivity than the single mutants. We concluded that the ubiquitin ligase and the ATPase activities of Rad5 are not epistatic. This implies that these two activities have independent functions, but it is not exclude the existence of a common function. We also intended to investigate the relationship of these two activities of Rad5 with *RAD18* and *RAD51* DNA repair pathways. Although in our previous results epistatic relationship was not manifested among *RAD5*, *RAD18* and *RAD51* genes with none of the tested mutagens, one could not exclude the possibility that either the ligase or the ATPase activity of Rad5 could interact with one of these pathways. To explore this possibility the epistatic relationship of *ring* and *atpase* mutants was analyzed on *rad18Δ* and on *rad51Δ* background. On these deletion backgrounds point mutants represented the similar sensitivity like on wild type background. These results suggest that domains of Rad5 could function in the same DNA repair pathway with both of the proteins Rad18 and Rad51. Or probably none of the domains function with these two proteins, only they function with one or more other proteins out of Rad18 and Rad51. This hypothesis was confirmed by the higher sensitivity of *rad5Δ/rad18Δ/rad51Δ* triple mutant strain than *rad5Δ/rad18Δ*, *rad18Δ/rad51Δ* and *rad18Δ/rad51Δ* double mutants. Although this sensitivity could be caused by other functions of the Rad5 (e.g. function of Hiran domain). To prove this theory we intended to test the sensitivity of the point mutants on *rad18Δ/rad51Δ* background.

To explore the role of the Hiran domain, we generated mutations in its conserved regions. Five from the twelve mutant strains showed sensitivity to DNA damaging agents (nitrogen-mustard and hydroxyurea). Two mutant strains from the five showed the same growth curve like wild type on mutagenic treatment if over-expression of proteins were induced. It means the low expression level of these two mutant proteins caused their sensitivity in our previous experiments. The other three were overexpressed, purified and tested *in vitro* in biochemical assays. The LI265,266RR mutant protein exhibited wild type activity while the GA177,178RR and the G183R mutants showed no activity neither in helicase nor in ubiquitin ligase assays. We concluded that the GA177,178 and the G183 parts of Hiran domain are likely to have a basic role in both of the two functions of Rad5. Nevertheless it is possible that these mutations modify the whole structure of the protein and it loses all of its activities. To answer this question more structural studies are needed with both wild type and mutant proteins.

We concluded that the role of Rad5 out of the *RAD18* pathway is none or just partially related to *RAD51*. In addition, the ubiquitin ligase and the ATPase/helicase activities of Rad5 have independent function from each other, and these functions are not exclusively func-