A Peptide-Based Dendrimer That Enhances the Splice-Redirecting Activity of PNA Conjugates in Cells

Fatouma Said Hassane,† Gabriela D. Ivanova,‡ Eleonora Bolewsk-Pedyczak,§ Rachida Abes,† Andrey A. Arzumanov,‡ Michael J. Gaît,‡ Bernard Lebleu,‡ and Jean Gariépy*†

UMR 5235 CNRS, Université Montpellier 2, Place Eugene Bataillon, 34095 Montpellier, France, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, United Kingdom, and Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, M5G 2M9, Ontario, Canada. Received February 16, 2009; Revised Manuscript Received June 1, 2009

The full therapeutic potential of oligonucleotide (ON)-based agents has been hampered by cellular delivery challenges. Cell-penetrating peptides (CPP) represent promising delivery vectors for nucleic acids, and their potential has recently been evaluated using a functional splicing redirection assay, which capitalizes on the nuclear delivery of splice-correcting steric-block ON analogues such as peptide nucleic acids (PNA). Despite encouraging in vitro and in vivo data with arginine-rich CPP-steric block conjugates, mechanistic studies have shown that entrapment within the endosome/lysosome compartment after endocytosis remains a limiting factor. Previous work from our group has shown that CPP oligomerization greatly improves cellular delivery and increases transfection of plasmid DNA. We now report the chemical synthesis and the evaluation of multivalent CPP—PNA constructs incorporating monomeric (p53mono) and dendrimer-like tetrameric (p53tet) forms of the p53 tetramerization domain containing peptide, a 10 arginine CPP domain (R10), and a splice redirecting PNA (PNA705). These CPP—PNA conjugates were termed R10p53tet-PNA705 and R10p53mono-PNA705, referring to their oligomerization state. The present study demonstrates that the splicing redirection efficiency of PNA705 is much greater in the context of the tetrameric R10p53tet-PNA705 construct than for the monomeric and occurs at nanomolar concentrations, demonstrating that multivalency is an important factor in delivering PNA into cells.

INTRODUCTION

Oligonucleotide (ON)-based agents have shown great potential in regulating gene expression and are routinely used to carry out functional genomics analyses. However, neither natural nucleic acids nor their chemically modified homologues are taken up efficiently by most cell types. While this challenge is easily circumvented through the use of transfection techniques in cultured cells, the systemic delivery of ONs in experimental animals remains a major limitation. Likewise, clinical trials with antisense ONs have resulted in mixed successes (1). Even very potent agents such as siRNAs have led to few clinical trials so far, due to a lack of efficient and nontoxic delivery vectors. Cell-penetrating peptides (CPP) represent good candidates for the intracellular delivery of nucleic acids with short oligoarginine motifs and peptides derived from the HIV Tat protein (Tat 48-60) and the Antennapedia homeodomain (Penetratin) being the most widely cited CPPs. The evaluation of CPPs in delivering functional ONs can best be achieved using the splicing redirection assay proposed by Kole (2). The assay is based on the splicing alteration in the β-globin gene in thallassemia. Intronic mutations (such as mutation 705 in intron 2) activate cryptic splice sites in this gene leading to a nonfunctional mRNA. This mutated intron has been introduced into the coding region of a reporter luciferase gene, and the construct has been stably transfected into HeLa cells. Masking the 705 mutated site with an antisense ON results in the reorientation of the splicing machinery toward intron elimination and to luciferase expression. The assay is easy to implement (assessing splicing by luminescence or by RT-PCR) and provides signals covering a high dynamic range. It has now been adopted by many groups in the field of drug delivery as a convenient way to compare the performance of various CPP carriers in delivering ON analogues (3-6). A broad range of ON analogues has been synthesized to date that do not recruit RNase H. However, peptide nucleic acids (PNA) and phosphorodiamidate morpholino oligomer (PMO) analogues are particularly suited as therapeutic agents since they are RNase H incompetent, are metabolically stable, and hybridize to RNA with high affinity and specificity. Initial assays with a panel of CPPs including Tat 48-60, Penetratin, oligo-Ag, or oligo-Lys have been disappointing, since no efficient splicing correction could be demonstrated in the absence of endosomolytic agents at submicromolar CPP–ON conjugate concentrations. By contrast, encouraging data have been obtained with an arginine-rich peptide named (RXXR)6-PNA or -PMO (where X stands for an unnatural aminohexanoyl moiety) (7), with R6-Penetratin-PNA (in which an Arg-rich tail has been appended to Penetratin) (δ), and with the subsequent serum-stable Pip-PNAs (δ). Dose-dependent and sequence-specific splicing correction has been achieved at micromolar or slightly lower concentrations in the absence of endosomolytic agents. Importantly, these PMO and PNA conjugates of Arg-rich CPPs also lead to splicing redirection in murine models of Duchenne muscular dystrophy (DMD) (10, 11). Such peptides are being evaluated for clinical applications, such as DMD, but it remains unclear at present whether the CPP-PMO doses required to be administered to humans will avoid potential liver and other toxic effects.

As far as mechanisms are concerned, it is now generally considered that CPP-PNA and PMO conjugates are taken up by endocytosis after binding to cell surface heparan sulfates.
Structure activity (SAR) and mechanistic studies currently in progress with conjugates based on both (RXR)4 and Pip CPP types have confirmed that entrapment within endocytic vesicles remains a major limitation. Studies of (RXR)-PMO intracellular distribution, in particular, clearly demonstrated that splicing redirection activity was due to the low amount of material that escaped from entrapment within endocytic vesicles.

The multivalent display of peptides harboring arginine- or lysine-containing CPP import sequences has been shown to dramatically enhance the cellular uptake levels of associated cargoes (12, 13). Specifically, synthetic branched peptides incorporating CPP sequences (oligomers) (12, 14, 15) as well as recombinant peptide constructs containing a self-assembling motif such as the p53 tetramerization domain (13, 16) have been shown to carry more efficiently drugs and chromophores into cells than the equivalent concentration of monomeric CPP homologues (15). Similarly, the condensation of such multivalent CPP dendrimers with plasmids results in particle complexes displaying excellent efficiency in entering targeted cells (14).

In this report, we discuss the chemical assembly and evaluation of multivalent CPP–PNA constructs incorporating monomeric and tetrameric forms of a p53tet domain containing peptide (p53tet), a 10 arginine CPP domain (R10), and a splice redirecting PNA (PNA705). These CPP–PNA conjugates were termed R10p53tet-PNA705 and R10p53mono-PNA705 constructs, referring to their oligomerization state. The present study demonstrates that multivalency is an important factor in delivering PNA into cells.

EXPERIMENTAL PROCEDURES

Synthesis of PNA. The N-terminal nitropyridyl (Npys) cysteine-containing PNA705 (NH2-Cys(NPys)-Lys-CCTCT-TACCTCAGTTACA-Lys-amide) was synthesized on a Liberty microwave peptide synthesizer (CEM) by the Fmoc/Bhoc solid-phase method as previously described using monomers obtained from Link Technologies Ltd. (Lamarkshire, Scotland) or Panagene Inc. (Daejeon, Korea), purified by HPLC, and analyzed by MALDI-TOF mass spectrometry on a Voyager DE Pro BioSpectrometry workstation as previously described (9, 17).

Synthesis and Characterization of R10p53 Peptides. The peptides R10p53tet (R10-GEYFTLQRGERFEMPREFNEALELKDAAQACGH6) and R10p53mono (R10GEYFTLQRGERFEMPREFNEALELKDAAQACGH6) were assembled from Fmoc solid-phase peptide synthesis as previously described (16). A single mutation of Leucine 344 to Proline (underlined residue) prevents tetramerization. A single cysteine residue was also introduced at their C-terminal end for the subsequent directional coupling of PNA. Peptides were purified to homogeneity by reverse-phase HPLC and their sequences were confirmed by MALDI-TOF mass spectrometry as described elsewhere (9, 17).

Peptide–PNA Conjugates. Single cysteine residues were also introduced in both PNA705 and R10p53 peptides to covalently link peptide and PNA via a disulfide bond (Table 1) as previously described, using a 2.5-fold excess of peptide component over Npys PNA component. Conjugate purifications were carried out by reverse-phase HPLC and analyzed by MALDI-TOF mass spectrometry as described elsewhere (9, 17).

Table 1. Sequences of PNA Conjugates

<table>
<thead>
<tr>
<th>Peptide</th>
<th>PNA705-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 tet</td>
<td>GCH6 CK-PNA705-K</td>
</tr>
<tr>
<td>p53 mono</td>
<td>GCH6 CK-PNA705-K</td>
</tr>
</tbody>
</table>

The PNA conjugates incorporating either monomeric or tetrameric R10p53 or (RXR)4 were incubated for 1 h at 37 °C in 1 mL optiMEM with exponentially growing subconfluent monolayers in DMEM medium (Gibco, Cergy-Pontoise, France) supplemented with 10% fetal bovine serum (FBS).

Splicing Redirection Assay. The PNA conjugates incorporating either monomeric or tetrameric R10p53 or (RXR)4 were incubated for 1 h at 37 °C in 1 mL optiMEM with exponentially growing HeLa pLuc 705 cells (1.75 × 10^6 cells/well seeded and cultivated overnight in 24 well plates). Cells were then washed twice with ice-cold PBS and incubation continued for 23 h in complete DMEM supplemented with 10% FBS. Cells were washed twice with ice-cold PBS and lysed with Reporter Lysis Buffer (Promega, Madison, WI, USA). The same protocol was used for HeLa pLuc 705 cells treated with 100 µM chloroquine (Figure 4B). Luciferase activity was measured using a Berthold Centro LB 960 luminometer (Berthold Technologies, Bad Wildbad, Germany) using the Luciferase Assay System substrate (Promega, Madison, WI, USA). Cellular protein concentrations were measured with the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and read using an ELISA plate reader (Dy Nametech MR 5000, Dy Nametech Laboratories, Chantilly, USA).
VA, USA) at 550 nm. Luciferase activities were expressed as relative luminescence units (RLU) per µg protein. All experiments were performed in triplicate. Each data point represents the average of three replicates.

**RT-PCR Analysis of SplicingRedirection.** Total RNA was extracted from cellular lysates obtained as described in the previous paragraph using the Tri-Reagent kit (Sigma, St. Quentin Fallavier, France) according to manufacturer’s instructions. The extracted RNA was amplified by RT-PCR (MJ Research PTC 200 Peltier Thermal Cycler) with forward primer 5′TTG ATA TGT GGA TTT CGA GTC GTC3′ and reverse primer 5′TGT CAA TCA GAG TGC TTT TGG CG3′. The products were analyzed by electrophoresis on a 2% agarose gel.

**Saponin Cell Permeabilization.** Exponentially growing HeLa pLuc705 cells (1.75 × 10^6 cells seeded and grown overnight in 24 well plates) were coincubated with the PNA conjugate and with 20 µg/mL of saponin for 30 min. The cells were then washed and incubation continued for 24 h in complete medium (DMEM plus 10% FBS). Cells were washed twice with ice-cold PBS, lysed with reporter lysis buffer, and processed as described above.

**RESULTS**

**Design and Characterization of p53tet Domain-Based PNA Delivery Peptides.** As described in the Introduction, the human p53tet tetramerization domain (residues 325–355; GEY-FTLQIRGRFERFMPRELNEALELKDQA) is able to self-assemble into a well-defined tetramer in solution and to enhance the cellular import of conjugated cationic CPPs, as compared to its monomeric counterpart (13, 16). Importantly, the condensation of NLS-R10-p53tet with luciferase reporter plasmid DNA led to complexes that increased the levels of luciferase expression in cells when compared to p53mono constructs (16).

In terms of designing CPP vectors better able to deliver steric-block ON analogues into cells, we have synthesized a new CPP construct in which an arginine-rich motif (R10) has been attached to the tetramerization domain (residues 325–355) of the human p53 protein. A cysteine was inserted near its C-terminus to covalently attach a PNA, while a short His6 tag was added for purification purposes (Table 1).

The synthetic peptide was chemically conjugated to a splice-redirecting 18-base-long PNA complementary to the 705 splice site (PNA 705), which we have used extensively in conjugation with other CPPs (9). In contrast to our previous PNP53 tet conjugates designed for plasmid delivery, no nuclear localization signal was included in the construct, since the molecular mass of the conjugate should be small enough to allow diffusion from the cytoplasm to the nuclei through the nuclear pores. R10p53tet-PNA705 conjugates were constructed by forming a disulfide bond between unique cysteine thiol groups present in the p53 peptide and the PNA705 (Table 1) (17). A monomeric PNA705 conjugated to a R10p53mono peptide was also synthesized to serve as a control. The monomeric form of the p53 tet domain was generated by introducing a single mutation (L344P) within its associated standard deviation derived from experiments performed in triplicate.

Figure 2. Comparison of R10p53tet-PNA705 with (RAhxR)4-PNA705 in the luciferase assay. HeLa pLuc 705 cells were incubated for 4 h with either R10p53tet-PNA705 or (RAhxR)4-PNA705 conjugate at the concentration of 1 µM. After treatment, cells were washed twice with 500 µL PBS, and incubation was continued for 20 h in the presence of complete medium (10% FBS plus DMEM). Luciferase expression was measured 20 h later and was expressed as RLU/µg protein. The control histogram bar represents cells untreated with peptide–PNA conjugates. Each histogram bar represents the averaged RLU/µg protein value with its associated standard deviation derived from experiments performed in triplicate.

these 10R-p53 peptides did not lead to cell toxicity even at concentrations of up to 25 µM (the highest concentration tested; ref 16).

**R10p53tet-PNA Is Very Effective at Correcting a Defective Luciferase Reporter Gene Located Inside Cells.** Our first objective was to establish if the construct R10p53tet-PNA705 CPP could enter HeLa pLuc705 cells and perform the splicing redirection function expected of the conjugated steric-block PNA705 oligomer leading to the expression of active luciferase (2). This construct was evaluated and compared to the PNA705 conjugate with the cationic CPP (RXX) (data not shown). The conjugate was prepared in OptiMEM medium to avoid technical issues arising from the absorption or proteolytic degradation of CPP peptides in serum. Specifically, the presence of serum has been shown by us and others to generally reduce the in vitro efficiency of CPP conjugates (5, 6, 19), an effect that does not correlate with their in vivo performance. For instance, (RAhxR)4 PMP conjugates performed poorly in vitro when prepared in serum-containing medium but have been used successfully in vivo in a murine model of Duchenne muscular dystrophy (9).

As presented in Figure 2, the treatment of HeLa pLuc705 cells with R10p53tet-PNA705 at 1 µM concentration in the absence of any transfection agent gave rise to an approximately 45-fold higher increase in luciferase signal when compared to the (RXX)–PNA705 conjugate. In order to monitor whether the increased efficiency of R10p53tet-PNA was due to peptide-based tetramerization, R10p53tet-PNA705 was also compared to its monomeric R10p53mono-PNA705 counterpart in a dose–response experiment. As expected, the tetrameric form was 10-fold more active in the redirection of luciferase splicing than its monomeric form at nanomolar concentrations (Figure 3A,B). Both peptide conjugates were soluble in the transfection media (OptiMEM), and no signs of toxicity were observed under the tested conditions, as assessed in terms of cell viability and protein levels (data not shown). Using the same experimental samples, it was also demonstrated that the cellular delivery of R10p53tet-PNA705 and R10p53mono-PNA705 constructs at submicromolar concentrations into HeLa pLuc705 cells resulted in
the correct splicing of the luciferase transcript at the RNA level (Figure 3C). In brief, treated cells were lysed, and aliquots were processed either for the luciferase luminescence assay or for an RT-PCR assay where the two forms (aberrant and correctly spliced) of luciferase mRNA were amplified. The appearance of the correctly spliced mRNA was dose-dependent and matched the decrease of the aberrantly spliced mRNA. Again, the splicing redirection event was significantly more pronounced for the tetrameric conjugate when compared to its monomeric counterpart.

Mechanism of Cellular Uptake. The mechanism by which cationic CPP–cargo conjugates enter cells is defined by the CPP valency/aggregation state within complexes (13, 16) and the concentration of the CPP/cargoes complexes outside cells. At extracellular CPP concentrations in the 5–100 µM range, cationic CPPs can enter cells via both endocytic and membrane-active mechanisms, which explains the co-observation of both energy-dependent endocytotic and energy-independent entry pathway(s) (20). However, the practicality of using CPPs in vivo as delivery vectors would favor their

Figure 3. Effect of peptide–PNA concentration on cellular gene correction event. Panels A and B. Dependency of cellular luciferase activity on extracellular peptide–PNA concentration as derived from two separate experiments. HeLa pLuc 705 cells were incubated for 1 h in medium containing R10p53tet-PNA705 (gray bars) or R10p53mono-PNA705 (white bars) conjugates at the indicated concentrations. Luciferase expression was measured 20 h later and expressed as RLU/µg protein. Each histogram bar represents the averaged RLU/µg protein value with its associated standard deviation derived from experiments performed in triplicate. Panel C. RT-PCR detection of correctly spliced transcripts in HeLa pLuc 705 cells at nanomolar concentrations of R10p53-PNA constructs. Lane 1, Nontreated cells (control); Lane 2, R10p53tet-PNA705 250 nM; Lane 3, R10p53tet-PNA705 500 nM; Lane 4, R10p53mono-PNA705 250 nM; Lane 5, R10p53mono-PNA705 500 nM. The RT-PCR experiment was performed with cellular lysates used for generating panel B. The upper band corresponds to the aberrantly spliced luciferase mRNA and the lower band to the correctly spliced mRNA.
use at low micromolar to submicromolar concentrations where their membrane active properties are not observed. Thus, a comparison of the mechanism of cell entry of the tetrameric and monomeric R10p53-PNA705 constructs seemed worthwhile. Therefore, cells were first preincubated at 37 °C (i.e., at a temperature that prevents any form of endocytosis) before being treated with the conjugates under the same conditions for 1 h. The results (Figure 4A) show a much-reduced splicing redirection activity (as measured by the level of luciferase activity) at low temperature for both monomeric and tetrameric constructs, which suggests an endocytotic mechanism of internalization occurring at a 1 µM concentration.

Since endosomal sequestration remains the principal limitation for the cellular delivery of ONs conjugated to CPPs that pass through the endocytotic route, we incubated cells with each form of R10p53-PNA705 in the presence or absence of chloroquine, a well-known endosomolytic agent (21, 22). Coincubation of R10p53_PNA705 conjugate with chloroquine treatment was found to have a much smaller effect on splicing redirection in the case of R10p53tet-PNA705.

**Effect of Saponin Treatment.** To circumvent the endocytotic pathway, cells were treated under mild conditions with saponin (20 µg/mL), a detergent which transiently permeabilizes the plasma membrane without damaging cells (23). Coincubation of R10p53mono-PNA705 conjugate with saponin gave the expected substantial augmentation of the splicing redirection activity (Figure 5) consistent with other cationic CPP-PNAs previously tested (7). However, the observed luciferase expression level remained lower than the value obtained for the tetrameric conjugate without permeabilization. The effect of saponin treatment on the cellular delivery of R10p53tet-PNA705 led to a lower splicing redirection activity comparable to splicing levels observed for the monomeric conjugate (Figure 4B), which supports the model that entrapment in endocytic vesicles is a limiting step in the cytoplasmic and nuclear release of such conjugates. Interestingly, chloroquine treatment was found to have a much smaller effect on splicing redirection in the case of R10p53tet-PNA705.

![Figure 4](image-url)

**Figure 4.** Mechanism of peptide–PNA internalization. Panel A: R10p53tet-PNA and R10p53mono-PNA conjugates are internalized by an energy-dependent mechanism. The splicing correction assay was used to assess the effect of temperature (energy dependency) on peptide–PNA delivery into cells. HeLa pLuc 705 cells were preincubated for 30 min at 4 °C (white bars) or at 37 °C (gray bars) and subsequently exposed to R10p53-PNA conjugates (1 µM) for 1 h under the same conditions. Panel B: Effect of chloroquine on the cytosolic delivery of peptide–PNA constructs leading to luciferase gene correction in HeLa pLuc 705 cells. Cells were incubated for 1 h with either R10p53tet-PNA705 or R10p53mono-PNA705 conjugates (1 µM) in the absence (white bars) or presence (gray bars) of 100 µM chloroquine. Luciferase expression was measured 23 h later and expressed as RLU/µg protein. Each histogram bar represents the averaged RLU/µg protein value with its associated standard deviation derived from experiments performed in triplicate.
conjugate. As shown in Figure 1 (lane C), detergents such as SDS do dissociate the tetramer.

**DISCUSSION**

Previous work has established that CPP oligomerization through the construction of peptide dendrimers can significantly improve cellular uptake and cytoplasmic delivery of arginine-rich CPPs. The potential of displaying multiple CPPs and their cargoes in the context of the p53 tetramerization domain was evaluated here using a well-characterized splicing redirection assay. The R10p53tet-PNA construct was approximately 45 times more efficient than the Pip2b-PNA construct (ref 9 and data not shown). These results are important, since the Pip series represented until now the most efficient cationic CPP-based PNA delivery vectors.

In order to verify whether the increased efficiency of the R10p53tet-PNA was effectively due to its tetrameric configuration, a monomeric version (R10p53mono-PNA) was engineered through the replacement of leucine 344 by a proline residue, a mutation known to prevent the tetramerization of p53 peptide. Splicing redirection was reduced by a factor of about 10 in keeping with the reduced transfection of a plasmid p53 peptide. Clues as to why both R10p53- and R10p53mono-PNA constructs remained slightly more active than the (RXR)4-PNA conjugate may be ascertained on this basis. The presence of chloroquine is thus not essential for cellular delivery using the R10p53tet-PNA construct. As highlighted in Figures 2–5, the tetrameric R10p53tet-PNA construct represents by far the most effective peptide-PNA705 construct to date in terms of correcting the genetic defect present in HeLa pLuc705 cells. This construct was able to deliver an effective dose of the PNA705 peptide nucleic acid to the cytosol of these cells when present outside HeLa pLuc705 cells at nanomolar concentrations. Somewhat unexpectedly, the R10p53mono-PNA construct remained slightly more active than the (RXR)4-PNA conjugate. As shown in Figure 1 (lane C), detergents such as SDS do dissociate the tetramer.

As highlighted in Figures 2–5, the tetrameric R10p53tet-PNA705 construct represents by far the most effective peptide-PNA705 construct to date in terms of correcting the genetic defect present in HeLa pLuc705 cells. This construct was able to deliver an effective dose of the PNA705 peptide nucleic acid to the cytosol of these cells when present outside HeLa pLuc705 cells at nanomolar concentrations. Somewhat unexpectedly, the R10p53mono-PNA construct remained slightly more active than the (RXR)4-PNA conjugate. As shown in Figure 1 (lane C), detergents such as SDS do dissociate the tetramer.
construct. Interestingly, (RAhxR)₄ PMO conjugates have been used in vivo (in the absence of the lysosomotropic amine agent chloroquine) and were able to promote exon skipping in the mdx mouse model of Duchenne muscular dystrophy (9). The constructs used in the present study are more efficient in vitro than these (RAhxR)₄ PMO constructs when compared in the same assay in the absence of chloroquine. Thus, we expect that they might be more efficient in vivo in the absence of chloroquine.

We thus attempted to deliberately bypass endocytosis by submitting cells to a brief saponin treatment under mild conditions, known to transiently permeabilize the plasma membrane with minimal alteration of cell physiology (23). As expected and in line with previous data from our group for other CPP–PMO/PMN conjugates (7), splicing correction by R10p53mono-PNA was increased in saponin-treated cells but did not reach the efficiency achieved by R10p53tet-PNA in untreated cells. One of the many possible explanations could be that the more bulky R10p53tet-PNA construct more efficiently masks the cryptic splice site and reorients the splicing machinery. Unexpectedly, splicing redirection was less efficient in saponin-treated cells for the R10p53tet-PNA construct. It might be due to a conversion of the tetrameric form into the monomeric form upon saponin treatment.

Altogether, our results confirm the potential of CPP oligomerization as a strategy to enhance the delivery of conjugated cargoes into cells. Specifically, the cellular delivery and splicing activity of PNA705 observed at nanomolar concentrations for the tetrameric R10p53-PNA construct is remarkable when compared to all other CPP strategies tested to date. This study provides a proof-of-concept for exploiting multivalent CPP strategies in the context of in vivo applications. Future work by our group will include the grafting of CPPs and cargoes onto simpler synthetic dendrimeric scaffolds.

ACKNOWLEDGMENT

This work was funded by IFCPAR (contract 3205-A) and AFM (contract 13345) grants to BL and by grants from the Canadian Breast Cancer Research Alliance in collaboration with the Canadian Cancer Society to JG. R. Abes is recipient of a predoctoral LNFCC fellowship and F. Said Hassane of a post doctoral AFM fellowship.

LITERATURE CITED


